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PATRICIA K HUMEWS
Typed or Printed Name of Person Mailing Paper or Fee

Patricia K HumeWS
Signature of Person Mailing Paper or Fee

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

ANDREW A. POTTER et al.

Serial No.: CON of 08/455,970

Group Art Unit: Unassigned

Filing Date: On Even Date Herewith

Examiner: Unassigned

Title: ENHANCED IMMUNOGENICITY USING LEUKOTOXIN CHIMERAS

**BLANKET PETITION FOR EXTENSION OF TIME AND
AUTHORIZATION TO CHARGE OR CREDIT DEPOSIT ACCOUNT**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

If a paper is untimely filed in this application or any file wrapper continuation application derived therefrom by applicant(s) or her/his/their representative, the Commissioner is hereby petitioned under 37 C.F.R. § 1.136(a) for the minimum extension of time required to make said paper timely. In the event a petition for extension of time is made under the provisions of this paragraph, the Commissioner is hereby requested to charge any fee required under 37 C.F.R. § 1.17(a)-(d) to Deposit Account No. 18-1648. This, however, is not authorization to pay the issue fee. **A duplicate copy of this sheet is attached.**

If a paper is concurrently or subsequently filed in this application or any file wrapper continuation application derived therefrom by applicant(s) or her/his/their representative and a fee under 37 C.F.R. §§ 1.16-1.17 is required to effect any amendment, petition or other action requested in said paper, the Commissioner is hereby requested to charge any deficiency in said fee, or credit any overpayment of said fee, to Deposit Account No. 18-1648. This, however, is not authorization to pay the issue fee.

Respectfully submitted,

Date: 11/24/97

By: Roberta L. Robins
Roberta L. Robins
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Respectfully submitted,

Date: 11/24/97

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**REQUEST FOR FILING
CONTINUATION/DIVISIONAL APPLICATION**

JCS30 U.S. PTO
11/24/97

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

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PATRICIA K. HIMENES
(Typed or Printed Name of Person Mailing Paper or Fee)

Patricia K. Himenes
(Signature of Person Mailing Paper or Fee)

Sir:

This is a request for filing a X continuation divisional application under 37 C.F.R. 1.60, of pending prior Application Serial No. 08/455,970 filed on May 31, 1995, which is a divisional of Application Serial No. 07/960,932 filed October 14, 1992 (now U.S. Patent No. 5,422,110), which is a continuation-in-part of Application Serial No. 07/779,171 filed October 16, 1991 (now abandoned), of Andrew A. Potter, Mark J. Redmond and Huw P.A. Hughes for ENHANCED IMMUNOGENICITY USING LEUKOTOXIN CHIMERAS.

1. X Enclosed is a copy of the latest inventor signed prior application, including the oath or declaration as originally filed. I hereby verify that the attached papers are a true copy of the latest inventor signed prior application serial no. 07/960,932 as originally filed on October 14, 1992, and further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.
2. X One verified statement(s) of small entity status
 enclosed.
X filed in prior application serial no. filed on (copy enclosed).

3. X The filing fee is calculated below (based on enclosed Preliminary Amendment):

A. Basic Application Fee		\$790
B. Total Claims 7 - 20 = 0	x \$22.00	0
C. Independent Claims 2 - 3 = 0	x \$82.00	0
D. If multiple claims present, add	\$270.00	0
E. Total Application Fee (Total of A, B, C & D)	=	790
F. If verified statement of small entity status is enclosed, reduce Total Application Fee by 50%		395
G. Application Fee Due (E - F)	=	395
H. Assignment Recording Fee of \$40.00 if assignment document is enclosed	\$40.00	0
I. TOTAL FEE (G + H)		\$395

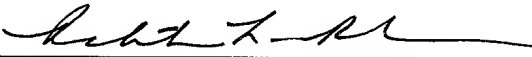
4. X The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 18-1648. **A duplicate copy of this sheet is enclosed.**
5. X A check to cover the \$ 395 application fee is attached.
6. X Cancel in this application original claims 2-36 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
7. X The inventor(s) of the invention being claimed in this application is (are): Andrew A. Potter, Mark J. Redmond and Huw P.A. Hughes.
8. This application is being filed by less than all the inventors named in the prior application. In accordance with 37 C.F.R. § 1.60(b), the Commissioner is requested to delete the name(s) of the following person or persons who are not inventors of the invention being claimed in this application:.
9. X Amend the specification by inserting before the first line the sentence:
This application is a continuation of U.S. Patent Application Serial No. 08/455,970 filed on May 31, 1995, which is a divisional of Application Serial No. 07/960,932 filed October 14, 1992 (now U.S. Patent No. 5,422,110), which is a continuation-in-part of Application Serial No. 07/779,171 filed October 16, 1991.
10. Transfer the drawings from the pending prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May only be used if signed by a person authorized by § 1.138 and before payment of issue fee.)

- a. ☐ New formal drawings are enclosed.
- b. ☐ Priority of application serial no. filed on in is claimed under 35 U.S.C. 119.

☐ The certified copy of the priority application
☐ is enclosed.
☐ has been filed in prior application serial no. filed on.
☐ has not yet been filed.

11. ☒ The prior application is assigned of record to University of Saskatchewan.
12. ☒ A preliminary amendment is enclosed.
13. ☒ Also enclosed: Blanket Petition for Extension of Time and Authorization to Charge or Credit Deposit Account, Sequence Listing, Certificate Regarding Sequence Listing, Information Disclosure Statement, PTO 1449, Formal Drawings with Transmittal Letter.
14. ☒ The power of attorney in the prior application is to Roberta L. Robins, Reg. No. 33,208.
- a. ☒ The power appears in the original papers in the prior application.
- b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
- c. ☒ Recognize the following individuals as Associate Attorney: Thomas P. McCracken, Reg. No. 38,548.
- d. ☒ Address all future communications to Roberta L. Robins at:
- ☒ ROBINS & ASSOCIATES
90 Middlefield Road, Suite 200
Menlo Park, CA 94025
phone: (650) 325-7812
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11/24/97
(date)


(Attorney of Record)
Roberta L. Robins
Reg. No. 33,208

Address of signator:

☐ inventor(s)
☐ assignee of complete interest
☒ attorney or agent of record

☐ filed under § 1.34(a)

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above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C.
20231.

PATRICIA K HIMERES
Typed or Printed Name of Person Mailing Paper or Fee

Patricia K Himeres
Signature of Person Mailing Paper or Fee

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

ANDREW A. POTTER et al.

Serial No.: CON of 08/455,970

Examiner: Unassigned

Filing Date: On Even Date Herewith

Art Unit: Unassigned

Title: ENHANCED IMMUNOGENICITY USING LEUKOTOXIN
CHIMERAS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to the examination of the above-captioned patent
application, applicant requests that it be amended as follows.

Amendment

In the Specification:

Please amend the specification as follows: Page 5, line 29, please delete "Figure 3 shows" and insert --Figures 3A through 3I (SEQ ID NOS:1 and 2) show--.

Page 6, line 1, after "Figure 4" please insert --(SEQ ID NOS:3-8)--.

Page 6, line 14, please delete "Figure 6 shows" and insert --Figures 6A through 6J (SEQ ID NOS:9 and 10) show--.

Page 6, line 27, please delete "Figure 8 shows" and insert --Figures 8A through 8J (SEQ ID NOS:11 and 12) show--.

Page 7, line 6, please delete "Figure 10 shows" and insert --Figures 10A through 10J (SEQ ID NOS:13 and 14) show--.

Page 8, line 22, after "Asp" please insert --(SEQ ID NO:15)--.

Page 9, line 22, please delete "Figure 3" and insert --Figures 3A through 3I (SEQ ID NOS:1 and 2)--.

Page 17, line 33, after "Figure 4" please insert --(SEQ ID NOS:3-8)--.

Page 18, lines 7-8, please delete "allowed U.S. Patent Application No. 07/539,236" and insert instead --U.S. Patent no. 5,212,156,--.

Page 35, line 9, please delete "Figure 3" and insert --Figures 3A through 3I (SEQ ID NOS:1 and 2)--.

Page 35, line 18, after "Figure 4" please insert --SEQ ID NOS:3-8--.

Page 35, line 33, please delete "6" and insert --Figures 6A through 6J (SEQ ID NO:9)--.

Page 35, line 33, please delete "8" and insert --Figures 8A through 8J (SEQ ID NO:11)--.

Page 35, line 33, please delete "10" and insert --Figures 10A through 10J (SEQ ID NO:13)--.

Please insert the Sequence Listing as new pages --42-74--.

In the Claims:

Please cancel claims 2-36, without prejudice and disclaimer.

Please add the following new claims:

--37. (New) A chimeric protein comprising a leukotoxin polypeptide coupled to a selected antigen.

38. (New) The chimeric protein of claim 37, wherein said leukotoxin polypeptide is coupled to somatostatin (SRIF), or an epitope thereof.

39. (New) The chimeric protein of claim 38, comprising the amino acid sequence of SEQ ID NO:10.

40. (New) The chimeric protein of claim 37, wherein said leukotoxin polypeptide is coupled to gonadotropin releasing hormone (GnRH), or an epitope thereof.

41. (New) The chimeric protein of claim 40, comprising the amino acid sequence of SEQ ID NO:12.

42. (New) The chimeric protein of claim 37, wherein said leukotoxin polypeptide is coupled to bovine rotavirus VP4, or an epitope thereof.

43. (New) The chimeric protein of claim 42, comprising the amino acid sequence of SEQ ID NO:14.--

Please renumber the claims pages from pages "42-47" to --74-79--.

In the Abstract:

Please renumber the abstract page from page "48" to --80--.

Remarks

Applicants, by way of the above amendment, are providing as a separate part of the disclosure, a "Sequence Listing" pursuant to 37 C.F.R. §§ 1.821-1.825. The specification and claims have been amended to refer to the sequence identification numbers and the subparts of the figures. Applicants are also submitting the Sequence Listing in paper copy. The undersigned hereby certifies that the computer readable form in this application is identical with that filed in parent application number 08/455,970, filed May 31, 1995. In accordance with 37 CFR §1.821(e), please use the last-filed computer readable form filed in that application as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application. A paper copy of the Sequence Listing is included herewith for incorporation into the specification.

Atty Dkt 9001-0016.01
CON of 08/455,970
PATENT

The specification has also been amended to include the now issued U.S. patent number which corresponds to the serial number previously cited.

Finally, claims 2-36 have been cancelled and new claims 37-43 added. The claims recite chimeric proteins comprising a leukotoxin polypeptide coupled to a selected antigen. Support for the new claims can be found in the claims as originally filed, as well as throughout the specification at, *inter alia*, page 17, lines 9-22.

Accordingly, no new matter has been added to the application by way of the above amendments.

Applicants respectfully request entry of the forgoing amendments prior to examination of the application.

Respectfully submitted,

Date: 11/24/97

By: Roberta L. Robins

Roberta L. Robins
Registration No. 33,208

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-1-

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Patricia K. Himenes
(Signature of Person Mailing Paper or Fee)

5

ENHANCED IMMUNOGENICITY USING LEUKOTOXIN CHIMERAS

Description

Cross-Reference to Related Application

10 This application is a continuation-in-part of
U.S. Patent Application Serial No. 07/779,171, filed 16
October 1991, from which priority is claimed pursuant to
35 USC §120 and which is hereby incorporated by reference
in its entirety.

15

Technical Field

The present invention relates generally to
immunological carrier systems. More particularly, the
invention pertains to leukotoxin-antigen chimeras which
20 demonstrate enhanced immunogenicity as compared to the
immunogenicity of the antigen alone.

Background of the Invention

Subunit vaccines are vaccines which are devoid
25 of intact pathogen cells. These vaccines are usually
composed of substantially purified antigens. Such
vaccines are generally preferable to compositions which
use attenuated or inactivated pathogens. However, many
subunit vaccines which include proteins, such as peptide
30 hormones and bacterial and viral antigens, require the
help of a carrier protein in order to elicit a strong
immune response. This is especially true for small
proteins or endogenous substances, such as hormones,
which are poorly immunogenic.

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The carrier serves to non-specifically stimulate T helper cell activity and to direct the antigen to the antigen presenting cell, where the antigen is processed and presented at the cell surface in the context of molecules of the major histocompatibility complex (MHC).

Several carrier systems have been developed for this purpose. For example, small peptide antigens are often coupled to protein carriers such as keyhole limpet haemocyanin (Bittle, J. L., et al., *Nature* (1982) 298:30-33), tetanus toxoid (Muller, G., et al., *Proc. Natl. Acad. Sci. U.S.A.* (1982) 79:569-573), ovalbumin, and sperm whale myoglobin, to produce an immune response. However, carriers may elicit strong immunity not relevant to the peptide antigen and this may inhibit the immune response to the peptide vaccine on secondary immunization (Schutze, M. P., et al., *J. Immun.* (1985) 135:2319-2322).

Antigen delivery systems have also been based on particulate carriers. For example, preformed particles have been used as platforms onto which antigens can be coupled and incorporated. Systems based on proteosomes (Lowell, G. H., et al., *Science* (1988) 240:800-802), immune stimulatory complexes (Morein, B., et al., *Nature* (1984) 308:457-460), and viral particles such as HBsAg (Neurath, A. R., et al., *Mol. Immunol.* (1989) 26:53-62) and rotavirus inner capsid protein (Redmond, M. J., et al., *Mol. Immunol.* (1991) 28:269-278) have been developed.

Other carrier systems have been devised using recombinantly produced chimeric proteins that self assemble into particles. For example, the yeast retrotransposon, Ty, encodes a series of proteins that assemble into virus like particles (Ty-VLPs; Kingsman, S. M., and A. J. Kingsman *Vacc.* (1988) 6:304-306). Foreign genes have been inserted into the TyA gene and expressed

[illegible][illegible][illegible][illegible]

response of antigens associated therewith has not heretofore been described.

Disclosure of the Invention

5 The present invention is based on the construction of novel gene fusions between the *P. haemolytica* leukotoxin gene and a nucleotide sequence encoding a selected antigen. These constructs produce a chimeric protein that displays enhanced immunogenicity
10 when compared to the immunologic reaction elicited by administration of the antigen alone.

 In one embodiment, the present invention is directed to an immunological carrier system comprising an immunogenic chimeric protein. The chimeric protein
15 comprises a leukotoxin polypeptide fused to a selected antigen, whereby the leukotoxin portion of the chimeric protein acts to increase the immunogenicity of the antigen. In particularly preferred embodiments, the selected antigen is somatostatin (SRIF), gonadotropin
20 releasing hormone (GnRH) or rotavirus viral protein 4 (VP4).

 Also disclosed are vaccine compositions comprising the chimeric proteins and a pharmaceutically acceptable vehicle and methods of using the same.

25 In another embodiment, the subject invention is directed to DNA constructs encoding the chimeric proteins. The DNA constructs comprise a first nucleotide sequence encoding a leukotoxin polypeptide operably linked to a second nucleotide sequence encoding the
30 selected antigen.

 In yet another embodiment, the subject invention is directed to expression cassettes comprised of (a) the DNA constructs above and (b) control sequences that direct the transcription of the construct whereby
35

the constructs can be transcribed and translated in a host cell.

In another embodiment, the invention is directed to host cells transformed with these expression
5 cassettes.

Another embodiment of the invention provides a method of producing a recombinant polypeptide. The method comprises (a) providing a population of host cells described above and (b) growing the population of cells
10 under conditions whereby the polypeptide encoded by the expression cassette is expressed.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

15

Brief Description of the Figures

Figure 1 depicts the structure of the leukotoxin gene of *P. haemolytica* cloned in *E. coli* (Plasmid pAA114).

20

Figure 2 depicts the structure of Plasmid pAA352 wherein *tac* is the hybrid *trp::lac* promoter from *E. coli*; *bla* represents the β -lactamase gene (ampicillin resistance); *ori* is the ColEI-based plasmid origin of replication; *lktA* is the *P. haemolytica* leukotoxin
25 structural gene; and *lacI* is the *E. coli* lac operon repressor. The direction of transcription/translation of the leukotoxin gene is indicated by the arrow. The size of each component is not drawn to scale.

Figure 3 shows the nucleotide sequence and
30 predicted amino acid sequence of leukotoxin 352 (LKT 352) from plasmid pAA352. Both the structural gene for LKT 352 and the sequences of the flanking vector regions are shown.

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Figure 4 shows the nucleotide sequences of SRIF, GnRH and bovine rotavirus VP4, used in the construction of the leukotoxin-antigen gene fusions.

Figure 5 shows the structure of Plasmid pAA496
5 carrying a leukotoxin-SRIF (LKT-SRIF) gene fusion wherein
tac is the hybrid trp::lac promoter from *E. coli*; bla
represents the β -lactamase gene (ampicillin resistance);
lktA is the *P. haemolytica* leukotoxin structural gene;
SRIF is the somatostatin structural gene; and lacI is the
10 *E. coli* lac operon repressor. The direction of
transcription/translation of the leukotoxin gene is
indicated by the arrow. The size of each component is
not drawn to scale.

Figure 6 depicts the nucleotide sequence and
15 predicted amino acid sequence of the LKT-SRIF chimeric
protein from pAA496.

Figure 7 shows the structure of Plasmid pAA502
carrying a leukotoxin-GnRH (LKT-GnRH) gene fusion wherein
tac is the hybrid trp::lac promoter from *E. coli*; bla
20 represents the β -lactamase gene (ampicillin resistance);
lktA is the *P. haemolytica* leukotoxin structural gene;
GnRH is the gonadotropin releasing hormone structural
gene; and lacI is the *E. coli* lac operon repressor. The
direction of transcription/translation of the leukotoxin
25 gene is indicated by the arrow. The size of each
component is not drawn to scale.

Figure 8 shows the nucleotide sequence and
predicted amino acid sequence of the LKT-GnRH chimeric
protein from pAA502.

Figure 9 depicts the structure of Plasmid
30 pAA501 carrying a leukotoxin-VP4 (LKT-VP4) gene fusion
wherein tac is the hybrid trp::lac promoter from *E. coli*;
bla represents the β -lactamase gene (ampicillin
resistance); lktA is the *P. haemolytica* leukotoxin
35 structural gene; VP4 is the bovine rotavirus viral

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protein 4 (232-255) structural gene; and lac1 is the *E. coli* lac operon repressor. The direction of transcription/translation of the leukotoxin gene is indicated by the arrow. The size of each component is not drawn to scale.

Figure 10 shows the nucleotide sequence and predicted amino acid sequence of the LKT-VP4 chimeric protein from pAA501.

10 Detailed Description

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, virology, recombinant DNA technology, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989); Maniatis, Fritsch & Sambrook, Molecular Cloning: A Laboratory Manual (1982); DNA Cloning, Vols. I and II (D.N. Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1984); Animal Cell Culture (R.K. Freshney ed. 1986); Immobilized Cells and Enzymes (IRL press, 1986); B. Perbal, A Practical Guide to Molecular Cloning (1984); the series, Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); and Handbook of Experimental Immunology, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., 1986, Blackwell Scientific Publications).

All patents, patent applications, and publications mentioned herein, whether supra or infra, are hereby incorporated by reference in their entirety.

A. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

5 An "antigen" refers to a molecule containing one or more epitopes that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is also used interchangeably with "immunogen." An antigen will include one or more
10 epitopes from a protein molecule, such as but not limited to, bacterial and viral proteins, as well as peptide hormones which elicit an immune response. Additionally, an antigen can comprise one or more identical or different immunogenic repeating sequences of a protein.
15 Specifically excluded from the definition for purposes of this application are cytokines such as interleukin-1 (IL1), interleukin-2 (IL2), interleukin-3 (IL3), interleukin-4 (IL4), and gamma-interferon (γ IFN).

 The term "leukotoxin polypeptide" intends a
20 polypeptide derived from a protein belonging to the family of molecules characterized by the carboxy-terminus consensus amino acid sequence Gly-Gly-X-Gly-X-Asp (Highlander et al., *DNA* (1989) 8:15-28), where X is Lys, Asp, Val or Asn. Such proteins include, among others,
25 leukotoxins derived from *P. haemolytica* and *Actinobacillus pleuropneumoniae*, as well as *E. coli* alpha hemolysin (Strathdee, C.A., and Lo, R.Y.C. *Infect. Immun.* (1987) 55:3233-3236; Lo, R.Y.C., *Can. J. Vet. Res.* (1990) 54:S33-S35; Welch, R.A., *Mol. Microbiol.* (1991) 5:521-
30 528). This family of toxins is known as the "RTX" family of toxins (Lo, R.Y.C., *Can. J. Vet. Res.* (1990) 54:S33-S35). In addition, the term "leukotoxin polypeptide" refers to a leukotoxin polypeptide which is chemically synthesized, isolated from an organism expressing the
35 same, or recombinantly produced. Furthermore, the term

intends an immunogenic protein having an amino acid sequence substantially homologous to a contiguous amino acid sequence found in the particular native leukotoxin molecule. Thus, the term includes both full-length and partial sequences, as well as analogs. Although native full-length leukotoxins display leukotoxic activity, the term "leukotoxin" also intends molecules which remain immunogenic yet lack the cytotoxic character of native leukotoxins. The nucleotide sequences and corresponding amino acid sequences for several leukotoxins are known. See, e.g., U.S. Patent Nos. 4,957,739 and 5,055,400; Lo et al., *Infect. Immun.* (1985) 50:667-67; Lo et al., *Infect. Immun.* (1987) 55:1987-1996; Strathdee, C.A., and Lo, R.Y.C., *Infect. Immun.* (1987) 55:3233-3236; Highlander et al., *DNA* (1989) 8:15-28; Welch, R.A., *Mol. Microbiol.* (1991) 5:521-528.

By "LKT 352" is meant a protein which is derived from the *lktA* gene present in plasmid pAA352 (Figure 2, ATCC Accession No. 68283). The nucleotide sequence and corresponding amino acid sequence of this gene are described in International Publication No. WO91/15237 and shown in Figure 3. The gene encodes a truncated leukotoxin, having 931 amino acids, which lacks the cytotoxic portion of the molecule. The derived LKT 352 is not necessarily physically derived from the sequence present in plasmid pAA352. Rather, it may be generated in any manner, including for example, by chemical synthesis or recombinant production. In addition, the amino acid sequence of the protein need only be substantially homologous to the depicted sequence. Thus, sequence variations may be present so long as the protein functions to enhance the immunogenicity of the antigen with which it is associated.

A "hapten" is a molecule containing one or more epitopes that does not stimulate a host's immune system to make a humoral or cellular response unless linked to a carrier.

5 The term "epitope" refers to the site on an antigen or hapten to which a specific antibody molecule binds. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site."

10 An "immunological response" to an antigen or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to the composition or vaccine of interest. Usually, such a response includes but is not limited to one or more of the following effects; the production of antibodies, B cells, 15 helper T cells, suppressor T cells, and/or cytotoxic T cells and/or $\gamma\delta$ T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest.

20 An "immunogenic protein" or "immunogenic amino acid sequence" is a protein or amino acid sequence, respectively, which elicits an immunological response in a subject to which it is administered.

25 A leukotoxin-antigen chimera displays "increased immunogenicity" when it possesses a greater capacity to elicit an immune response than the corresponding antigen alone. Such increased immunogenicity can be determined by administering the particular leukotoxin-antigen and antigen controls to animals and comparing antibody titers against the two 30 using standard assays such as radioimmunoassays and ELISAs, well known in the art.

35 By "carrier system" is meant a system which includes a molecule that serves to increase the immunogenicity of an antigen administered therewith, as defined above. Without being bound by any particular

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theory, the molecule may function to increase the immunogenicity of the antigen by presenting the same to cells of the immune system, such as antigen presenting cells, macrophages, follicular dendritic cells, B cells and T cells; or by stimulating the immune system to respond at a level greater than that observed when the antigen is administered alone.

By "subunit antigen composition" is meant a composition containing at least one immunogenic polypeptide, but not all antigens, derived from or homologous to an antigen from a pathogen of interest. Such a composition is substantially free of intact pathogen cells or particles. Generally, a "subunit antigen composition" is prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen, or recombinant analogs thereof.

The term "protein" is used herein to designate a naturally occurring polypeptide. The term "polypeptide" is used in its broadest sense, i.e., any polymer of amino acids (dipeptide or greater) linked through peptide bonds. Thus, the term "polypeptide" includes proteins, oligopeptides, protein fragments, analogs, muteins, fusion proteins and the like.

"Native" proteins or polypeptides refer to proteins or polypeptides recovered from a source occurring in nature. Thus, the term "native leukotoxin" would include naturally occurring leukotoxin and fragments thereof.

"Recombinant" polypeptides refer to polypeptides produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide. "Synthetic" polypeptides are those prepared by chemical synthesis.

5 A "rotavirus VP6 protein" refers to the art-
recognized major viral protein of the inner capsid from
any species or strain within the family Reoviridae. See,
e.g., Kapikian et al., 1985. Examples of rotavirus
10 strains from which the VP6 protein can be isolated and
employed in the present invention include, but are not
limited to, Simian SA-11, human D rotavirus, bovine UK
rotavirus, human Wa or W rotavirus, human DS-1 rotavirus,
rhesus rotavirus, the "O" agent, bovine NCDV rotavirus,
15 human S2 rotavirus, human KUN rotavirus, human 390
rotavirus, human P rotavirus, human M rotavirus, human
Walk 57/14 rotavirus, human Mo rotavirus, human Ito
rotavirus, human Nemoto rotavirus, human YO rotavirus,
human McM2 rotavirus, rhesus monkey MMU18006 rotavirus,
20 canine CU-1 rotavirus, feline Taka rotavirus, equine H-2
rotavirus, human St. Thomas No. 3 and No. 4 rotaviruses,
human Hosokawa rotavirus, human Hochi rotavirus, porcine
SB-2 rotavirus, porcine Gottfried rotavirus, porcine
SB-1A rotavirus, porcine OSU rotavirus, equine H-1
25 rotavirus, chicken Ch.2 rotavirus, turkey Ty.1 rotavirus,
bovine C486 rotavirus, and strains derived from them.
Thus the present invention encompasses the use of VP6
from any rotavirus strain, whether from subgroup I,
subgroup II, or any as yet unidentified subgroup, as well
30 as from any of the serotypes 1-7, as well as any as yet
unidentified serotypes. Such VP6 proteins can be used as
immunologic carriers of polypeptides. These carrier
molecules comprise amino acid sequences of rotavirus VP6
amino acid sequences which are unique to the class, or
35 any member of the class, of VP6 polypeptides. Such
unique sequences of VP6 proteins are referred to as a
"rotavirus VP6 inner capsid protein amino acid Sequence."
VP6 carriers are further disclosed in U.S. Patent No.
5,071,651, incorporated herein by reference in its
entirety.

A carrier that is "substantially homologous to a rotavirus VP6 inner capsid protein or a functional fragment thereof" is one in which at least about 85%, preferably at least about 90%, and most preferably at least about 95%, of the amino acids match over a defined length of the molecule. A "functional fragment" of a rotavirus VP6 inner capsid protein is a fragment with the capability of acting as a carrier molecule for the novel chimeric proteins of the instant invention.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo* or *in vitro*; i.e., capable of replication under its own control.

A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "double-stranded DNA molecule" refers to the polymeric form of deoxyribonucleotides in a double-stranded helix, both relaxed and supercoiled. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having the sequence homologous to the mRNA).

A DNA "coding sequence" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide *in vivo* or *in vitro* when placed under the

control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding
5 sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding
10 sequence.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present
15 invention, the promoter sequence is bound at the 3' terminus by the translation start codon (ATG) of a coding sequence and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above
20 background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eucaryotic promoters will often, but
25 not always, contain "TATA" boxes and "CAT" boxes. Procaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

DNA "control sequences" refers collectively to promoter sequences, ribosome binding sites,
30 polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the transcription and translation of a coding sequence in a host cell.

A coding sequence is "operably linked to" another coding sequence when RNA polymerase will transcribe the two coding sequences into mRNA, which is then translated into a chimeric polypeptide encoded by the two coding sequences. The coding sequences need not be contiguous to one another so long as the transcribed sequence is ultimately processed to produce the desired chimeric protein.

A control sequence "directs the transcription" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed, or is capable of transformation, by an exogenous DNA sequence.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) to chromosomal DNA making up the genome of the cell. In procaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eucaryotic cells, a stably transformed cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eucaryotic cell to establish cell lines or clones comprised of a population of daughter cell containing the exogenous DNA.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

A composition containing A is "substantially free of" B when at least about 85% by weight of the total of A + B in the composition is A. Preferably, A comprises at least about 90% by weight of the total of A

+ B in the composition, more preferably at least about 95%, or even 99% by weight.

The term "treatment" as used herein refers to either (i) the prevention of infection or reinfection (prophylaxis), or (ii) the reduction or elimination of symptoms or the disease of interest (therapy).

B. General Methods

Central to the instant invention is the discovery that leukotoxin polypeptides, when coupled to selected antigens, are able to increase the immunogenicity of the antigen as compared to the immunogenicity of the antigen when presented alone. Thus, leukotoxin polypeptides can act as carrier proteins for the presentation of a desired antigen to the immune system. Accordingly, the chimeric proteins can be formulated into vaccine compositions which provide enhanced immunogenicity to the antigen presented therewith. The fusion of the leukotoxin gene to the selected antigen further functions to facilitate purification of the chimeric protein from cells expressing the same.

The leukotoxin carrier is especially useful for the presentation of small or endogenous peptide antigens, including peptide hormones, and bacterial and viral antigens, which typically elicit poor immune responses when presented without the aid of a carrier. Exemplified herein are leukotoxin chimeras which include leukotoxin fused to small peptide hormones -- somatostatin (SRIF) and gonadatropin releasing hormone (GnRH). SRIF-14 has 14 amino acids and GnRH possesses 10 amino acids. The nucleotide sequences of SRIF and GnRH are depicted in Figure 4. Because the sequences are relatively short, they can easily be generated using synthetic techniques, as described further below. Because these hormones are

small in size and are endogenous to several mammals such as humans, bovines etc., these substances require the use of carrier proteins in order to elicit an adequate immune response in such mammals. Immunization with these
5 hormones can regulate growth rate, lactation and reproductive efficiency. A detailed discussion of SRIF can be found in allowed U.S. Patent Application No. 07/539,236, filed 18 June 1990, which is incorporated herein by reference in its entirety. GnRH is further
10 discussed in U.S. Patent No. 4,975,420, incorporated herein by reference in its entirety.

Also exemplified herein is a chimera comprised of leukotoxin and bovine rotavirus viral protein 4 (VP4). VP4 (molecular weight 86,719), functions as the viral
15 hamagglutinin and forms the spike-like projections protruding from the surface of the virus. Antibodies capable of neutralizing the virus bind to the tip of the spike. VP4 appears to play a major role in viral attachment during infection. The nucleotide sequence of
20 VP4 is depicted in Figure 4. For a further discussion of rotavirus infection and VP4, see, Redmond, M.J. et al. in Viral Diseases (Ed. E. Kurstak, Marcel Dekker, New York, 1991, pp. 387-404); and International Publication No. WO/9207941, published 14 May 1992, both incorporated
25 herein by reference in their entirety. Although the invention is described with respect to these particular proteins, leukotoxin polypeptides, or proteins functionally equivalent and substantially homologous thereto, can be easily fused to other antigens, based on
30 the disclosure herein, in order to increase the immunogenicity thereof.

The leukotoxin-antigen complex can be conveniently produced recombinantly as a chimeric protein. The antigen portion of the chimera can be fused

either 5' or 3' to the leukotoxin portion of the molecule.

Actively growing cells of *P. haemolytica* have been shown to secrete leukotoxin which can be cloned, the gene encoding the same isolated, and fused with a gene encoding a desired antigen, using techniques well known in the art. The resulting chimeric proteins can be expressed and used to immunize subjects against the particular antigen fused to leukotoxin.

The nucleotide sequence coding for full-length *P. haemolytica* A1 leukotoxin has been determined. See, e.g., Lo, R.Y.C. *Infect. Immun.* (1987) 55:1987-1996; U.S. Patent No. 5,055,400, incorporated herein by reference in its entirety. *P. haemolytica* leukotoxin can be produced using recombinant techniques and purified from, for example, bacterial cells. The leukotoxin can also be purified from native bacteria using immunoadsorbent chromatography.

Similarly, the coding sequences for numerous antigens are known or can be determined. Again, these antigens can be purified using standard techniques.

Purification of the above proteins, using standard techniques including those described herein, permits the sequencing of the same by any of the various methods known to those skilled in the art. For example, the amino acid sequences can be determined by repetitive cycles of Edman degradation, followed by amino acid analysis by HPLC. Other methods of amino acid sequencing are also known in the art. Furthermore, fragments of the proteins can be tested for biological activity and active epitopes used in compositions in lieu of the entire protein.

Once the amino acid sequences are determined, oligonucleotide probes which contain the codons for a portion of the determined amino acid sequences can be

prepared and used to screen DNA libraries for genes encoding the subject proteins. The basic strategies for preparing oligonucleotide probes and DNA libraries, as well as their screening by nucleic acid hybridization, are well known to those of ordinary skill in the art. See, e.g., DNA Cloning: Vol. I, supra; Nucleic Acid Hybridization, supra; Oligonucleotide Synthesis, supra; T. Maniatis et al., supra.

First, a DNA library is prepared. The library can consist of genomic DNA from *P. haemolytica* (for the isolation of the leukotoxin gene) or from appropriate cells or viruses (for the isolation of the desired antigen gene). Once the library is constructed, oligonucleotides to probe the library are prepared and used to isolate the gene encoding the desired protein. The oligonucleotides are synthesized by any appropriate method. The particular nucleotide sequences selected are chosen so as to correspond to the codons encoding a known amino acid sequence from the desired protein. Since the genetic code is degenerate, it will often be necessary to synthesize several oligonucleotides to cover all, or a reasonable number, of the possible nucleotide sequences which encode a particular region of the protein. Thus, it is generally preferred in selecting a region upon which to base the probes, that the region not contain amino acids whose codons are highly degenerate. In certain circumstances, one of skill in the art may find it desirable to prepare probes that are fairly long, and/or encompass regions of the amino acid sequence which would have a high degree of redundancy in corresponding nucleic acid sequences, particularly if this lengthy and/or redundant region is highly characteristic of the protein of interest. It may also be desirable to use two probes (or sets of probes), each to different regions of the gene, in a single hybridization experiment.

Automated oligonucleotide synthesis has made the preparation of large families of probes relatively straightforward. While the exact length of the probe employed is not critical, generally it is recognized in the art that
5 probes from about 14 to about 20 base pairs are usually effective. Longer probes of about 25 to about 60 base pairs are also used.

The selected oligonucleotide probes are labeled with a marker, such as a radionucleotide or biotin, using
10 standard procedures. The labeled set of probes is then used in the screening step, which consists of allowing the single-stranded probe to hybridize to isolated ssDNA from the library, according to standard techniques. Either stringent or permissive hybridization conditions
15 could be appropriate, depending upon several factors, such as the length of the probe and whether the probe is derived from the same species as the library, or an evolutionarily close or distant species. The selection of the appropriate conditions is within the skill of the
20 art. See, generally, Nucleic Acid hybridization, supra. The basic requirement is that hybridization conditions be of sufficient stringency so that selective hybridization occurs; i.e., hybridization is due to a sufficient degree of nucleic acid homology (e.g., at least about 75%), as
25 opposed to nonspecific binding. Once a clone from the screened library has been identified by positive hybridization, it can be confirmed by restriction enzyme analysis and DNA sequencing that the particular library insert contains a gene for the desired protein.

Alternatively, DNA sequences encoding the
30 proteins of interest can be prepared synthetically rather than cloned. The DNA sequence can be designed with the appropriate codons for the particular amino acid sequence. In general, one will select preferred codons
35 for the intended host if the sequence will be used for

expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature* (1981) 292:756; Nambair et al. *Science* (1984) 223:1299; Jay et al. *J. Biol. Chem.* (1984) 259:6311.

Once coding sequences for the desired proteins have been prepared or isolated, they can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage lambda (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-*E. coli* gram-negative bacteria), pHV14 (*E. coli* and *Bacillus subtilis*), pBD9 (*Bacillus*), pIJ61 (*Streptomyces*), pUC6 (*Streptomyces*), YIp5 (*Saccharomyces*), YCp19 (*Saccharomyces*) and bovine papilloma virus (mammalian cells). See, generally, DNA Cloning: Vols. I & II, supra; T. Maniatis et al., supra; B. Perbal, supra.

Suitable restriction enzymes can then be employed to isolate the appropriate antigen gene or leukotoxin gene and these sequences can be ligated together and cloned to form a leukotoxin-antigen fusion gene.

The fusion gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the chimeric protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding

sequence may or may not contain a signal peptide or leader sequence. The chimeric proteins of the present invention can be expressed using, for example, native *P. haemolytica* promoter, the *E. coli* tac promoter or the protein A gene (spa) promoter and signal sequence.
5 Leader sequences can be removed by the bacterial host in post-translational processing. See, e.g., U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397.

10 In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a
15 gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

20 An expression vector is constructed so that the particular fusion coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the
25 control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the sequences encoding the particular chimeric protein of interest may be desirable to achieve this end. For example, in some
30 cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to
35 insertion into a vector, such as the cloning vectors

described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

5 In some cases, it may be desirable to add sequences which cause the secretion of the polypeptide from the host organism, with subsequent cleavage of the secretory signal. It may also be desirable to produce mutants or analogs of the chimeric proteins of interest.
10 Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed
15 mutagenesis, are well known to those skilled in the art. See, e.g., T. Maniatis et al., supra; DNA Cloning, Vols. I and II, supra; Nucleic Acid Hybridization, supra.

A number of procaryotic expression vectors are known in the art. See, e.g., U.S. Patent Nos. 4,440,859;
20 4,436,815; 4,431,740; 4,431,739; 4,428,941; 4,425,437; 4,418,149; 4,411,994; 4,366,246; 4,342,832; see also U.K. Patent Applications GB 2,121,054; GB 2,008,123; GB 2,007,675; and European Patent Application 103,395. Yeast expression vectors are also known in the art. See,
25 e.g., U.S. Patent Nos. 4,446,235; 4,443,539; 4,430,428; see also European Patent Applications 103,409; 100,561; 96,491.

Depending on the expression system and host selected, the proteins of the present invention are
30 produced by growing host cells transformed by an expression vector described above under conditions whereby the protein of interest is expressed. The chimeric protein is then isolated from the host cells and purified. If the expression system secretes the protein into growth
35 media, the protein can be purified directly from the

media. If the protein is not secreted, it is isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

5 An alternative method to identify proteins of the present invention is by constructing gene libraries, using the resulting clones to transform *E. coli* and pooling and screening individual colonies using polyclonal serum or monoclonal antibodies to the desired
10 antigen.

 The chimeric proteins of the present invention may also be produced by chemical synthesis such as solid phase peptide synthesis, using known amino acid sequences or amino acid sequences derived from the DNA sequence of
15 the genes of interest. Such methods are known to those skilled in the art. Chemical synthesis of peptides may be preferable if a small fragment of the antigen in question is capable of raising an immunological response in the subject of interest.

20 The proteins of the present invention or their fragments can be used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal, (e.g., mouse, rabbit, goat, horse, etc.) is immunized with an antigen of the present
25 invention, or its fragment, or a mutated antigen. Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies is used, the polyclonal antibodies can be purified by immunoaffinity chromatography, using
30 known procedures.

 Monoclonal antibodies to the proteins of the present invention, and to the fragments thereof, can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by
35 hybridomas is well known. Immortal antibody-producing

cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al.,
5 Hybridoma Techniques (1980); Hammerling et al.,
Monoclonal Antibodies and T-cell Hybridomas (1981); Kennett et al., Monoclonal Antibodies (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,452,570; 4,466,917; 4,472,500, 4,491,632;
10 and 4,493,890. Panels of monoclonal antibodies produced against the antigen of interest, or fragment thereof, can be screened for various properties; i.e., for isotype, epitope, affinity, etc. Monoclonal antibodies are useful in purification, using immunoaffinity
15 techniques, of the individual antigens which they are directed against.

Animals can be immunized with the compositions of the present invention by administration of the chimeric protein, or an active fragment thereof, or an
20 analog thereof. The chimeric protein can consist of leukotoxin fused to an epitope of the desired antigen, as defined above. Thus, if the fragment or analog of the fusion protein is used, it will include the amino acid sequence of leukotoxin, or a fragment of the same which
25 interacts with the immune system to increase the immunogenicity of the antigen or epitope thereof, linked to the antigen of interest.

Prior to immunization, it may be desirable to further increase the immunogenicity of the particular
30 chimeric protein, or an analog of the protein, or particularly fragments of the protein. This can be accomplished in any one of several ways known to those of skill in the art. For example, the antigenic peptide may be administered linked to a carrier, in addition to the
35 leukotoxin carrier. For example, a fragment may be

conjugated with a macromolecular carrier. Suitable carriers are typically large, slowly metabolized macromolecules such as: proteins; polysaccharides, such as sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, and other proteins well known to those skilled in the art.

The protein substrates may be used in their native form or their functional group content may be modified by, for example, succinylation of lysine residues or reaction with Cys-thiolactone. A sulfhydryl group may also be incorporated into the carrier (or antigen) by, for example, reaction of amino functions with 2-iminothiolane or the N-hydroxysuccinimide ester of 3-(4-dithiopyridyl) propionate. Suitable carriers may also be modified to incorporate spacer arms (such as hexamethylene diamine or other bifunctional molecules of similar size) for attachment of peptides.

Other suitable carriers for the chimeric proteins of the present invention include VP6 polypeptides of rotaviruses, or functional fragments thereof, as disclosed in U.S. Patent No. 5,071,651, and incorporated herein by reference. Also useful is a fusion product of a viral protein and the subject leukotoxin-antigen immunogen made by methods disclosed in U.S. Patent No. 4,722,840. Still other suitable carriers include cells, such as lymphocytes, since presentation in this form mimics the natural mode of presentation in the subject, which gives rise to the immunized state. Alternatively, the fusion proteins of the present invention may be coupled to erythrocytes, preferably the subject's own erythrocytes. Methods of coupling peptides

to proteins or cells are known to those of skill in the art.

The novel chimeric proteins of the instant invention can also be administered via a carrier virus which expresses the same. Carrier viruses which will find use with the instant invention include but are not limited to the vaccinia and other pox viruses, adenovirus, and herpes virus. By way of example, vaccinia virus recombinants expressing the novel chimeric proteins can be constructed as follows. The DNA encoding the particular leukotoxin-antigen chimeric protein is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the instant chimeric protein into the viral genome. The resulting TKrecombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

It is also possible to immunize a subject with a protein of the present invention, or an immunogenic fragment thereof, or an analog thereof, which is administered alone, or mixed with a pharmaceutically acceptable vehicle or excipient. Typically, vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles. The active immunogenic ingredient is often mixed with vehicles containing excipients which are pharmaceutically acceptable and compatible with the ac-

tive ingredient. Suitable vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccine. Adjuvants may include for example, muramyl dipeptides, avridine, aluminum hydroxide, oils, saponins and other substances known in the art. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 15th edition, 1975. The composition or formulation to be administered will, in any event, contain a quantity of the protein adequate to achieve the desired immunized state in the subject being treated.

Additional vaccine formulations which are suitable for other modes of administration include suppositories and, in some cases, aerosol, intranasal, oral formulations, and sustained release formulations. For suppositories, the vehicle composition will include traditional binders and carriers, such as, polyalkaline glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), preferably about 1% to about 2%. Oral vehicles include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium, stearate, sodium saccharin cellulose, magnesium carbonate, and the like. These oral vaccine compositions may be taken in the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders, and contain from about 10% to about 95% of the active ingredient, preferably about 25% to about 70%.

Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

Controlled or sustained release formulations are made by incorporating the chimeric protein into carriers or vehicles such as liposomes, nonresorbable impermeable polymers such as ethylenevinyl acetate copolymers and Hytrel® copolymers, swellable polymers such as hydrogels, or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make resorbable sutures. The chimeric proteins can also be presented using implanted mini-pumps, well known in the art.

Furthermore, the chimeric proteins (or complexes thereof) may be formulated into vaccine compositions in either neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the active polypeptides) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

5 To immunize a subject, the polypeptide of
interest, or an immunologically active fragment thereof,
is administered parenterally, usually by intramuscular
injection in an appropriate vehicle. Other modes of
10 administration, however, such as subcutaneous,
intravenous injection and intranasal delivery, are also
acceptable. Injectable vaccine formulations will contain
an effective amount of the active ingredient in a
vehicle, the exact amount being readily determined by one
15 skilled in the art. The active ingredient may typically
range from about 1% to about 95% (w/w) of the
composition, or even higher or lower if appropriate. The
quantity to be administered depends on the animal to be
treated, the capacity of the animal's immune system to
20 synthesize antibodies, and the degree of protection
desired. With the present vaccine formulations,
approximately 1 μ g to 1 mg, more generally 5 μ g to 100 μ g
of antigen per ml of injected solution, should be
adequate to raise an immunological response when
25 administered. Other effective dosages can be readily
established by one of ordinary skill in the art through
routine trials establishing dose response curves. The
subject is immunized by administration of the particular
antigen or fragment thereof, or analog thereof, in at
30 least one dose, and preferably two doses. Moreover, the
animal may be administered as many doses as is required
to maintain a state of immunity.

Below are examples of specific embodiments for
carrying out the present invention. The examples are of-
35 fered for illustrative purposes only, and are not
intended to limit the scope of the present invention in
any way.

Deposits of Strains Useful in Practicing the Invention

A deposit of biologically pure cultures of the following strains was made with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland.

5 The accession number indicated was assigned after successful viability testing, and the requisite fees were paid. Access to said cultures will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR 1.14
10 and 35 USC 122. All restriction on availability of said cultures to the public will be irrevocably removed upon the granting of a patent based upon the application. Moreover, the designated deposits will be maintained for a period of thirty (30) years from the date of deposit,
15 or for five (5) years after the last request for the deposit; or for the enforceable life of the U.S. patent, whichever is longer. Should a culture become nonviable or be inadvertently destroyed, or, in the case of plasmid-containing strains, lose its plasmid, it will be
20 replaced with a viable culture(s) of the same taxonomic description.

These deposits are provided merely as a convenience to those of skill in the art, and are not an admission that a deposit is required under 35 USC §112.
25 The nucleic acid sequences of these plasmids, as well as the amino sequences of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with the description herein. A license may be required to make, use, or sell the
30 deposited materials, and no such license is hereby granted.

<u>Strain</u>	<u>Deposit Date</u>	<u>ATCC No.</u>
P. haemolytica serotype 1 B122	February 1, 1989	53863
pAA352 in <i>E. coli</i> W1485	March 30, 1990	68283

C. Experimental

Materials and Methods

Enzymes were purchased from commercial sources,
5 and used according to the manufacturers' directions.
Radionucleotides and nitrocellulose filters were also
purchased from commercial sources.

In the cloning of DNA fragments, except where
noted, all DNA manipulations were done according to
10 standard procedures. See Sambrook et al., supra.
Restriction enzymes, T₄ DNA ligase, *E. coli*, DNA
polymerase I, Klenow fragment, and other biological
reagents were purchased from commercial suppliers and
used according to the manufacturers' directions. Double-
15 stranded DNA fragments were separated on agarose gels.

cDNA and genomic libraries were prepared by
standard techniques in pUC13 and the bacteriophage lambda
gt11, respectively. See DNA CLONING: Vols I and II,
supra.

20 *P. haemolytica* biotype A, serotype 1 ("A1")
strain B122 was isolated from the lung of a calf which
died of pneumonic pasteurellosis and was stored at -70°C
in defibrinated blood. Routine propagation was carried
out on blood agar plates or in brain heart infusion broth
25 (Difco Laboratories, Detroit, MI) supplemented with 5%
(v/v) horse serum (Gibco Canada Ltd., Burlington,
Canada). All cultures were incubated at 37°C.

Example 1

30 Isolation of *P. haemolytica* Leukotoxin Gene

To isolate the leukotoxin gene, gene libraries
of *P. haemolytica* A1 (strain B122) were constructed using
standard techniques. See, Lo et al., *Infect. Immun.*,
supra; DNA CLONING: Vols. I and II, supra; and
35 T. MANIATIS et al., supra. A genomic library was

constructed in the plasmid vector pUC13 and a DNA library
constructed in the bacteriophage lambda gt11. The
resulting clones were used to transform *E. coli* and
individual colonies were pooled and screened for reaction
5 with serum from a calf which had survived a *P.*
haemolytica infection and that had been boosted with a
concentrated culture supernatant of *P. haemolytica* to
increase anti-leukotoxin antibody levels. Positive
colonies were screened for their ability to produce
10 leukotoxin by incubating cell lysates with bovine
neutrophils and subsequently measuring release of lactate
dehydrogenase from the latter.

Several positive colonies were identified and
these recombinants were analyzed by restriction
15 endonuclease mapping. One clone appeared to be identical
to a leukotoxin gene cloned previously. See, Lo et al.,
Infect. Immun., supra. To confirm this, smaller
fragments were recloned and the restriction maps
compared. It was determined that approximately 4
20 kilobase pairs of DNA had been cloned. Progressively
larger clones were isolated by carrying out a chromosome
walk (5' to 3' direction) in order to isolate full-length
recombinants which were approximately 8 kb in length.
The final construct was termed pAA114. This construct
25 contained the entire leukotoxin gene sequence. The
structure of this plasmid is shown in Figure 1.

lktA, a MaeI restriction endonuclease fragment
from pAA114 which contained the entire leukotoxin gene,
was treated with the Klenow fragment of DNA polymerase I
30 plus nucleotide triphosphates and ligated into the SmaI
site of the cloning vector pUC13. This plasmid was named
pAA179. From this, two expression constructs were made
in the ptac-based vector pGH432: lacI digested with SmaI.
One, pAA342, consisted of the 5'-AhaIII fragment of the
35 lktA gene while the other, pAA345, contained the entire

MaeI fragment described above. The clone pAA342 expressed a truncated leukotoxin peptide at high levels while pAA345 expressed full length leukotoxin at very low levels. Therefore, the 3' end of the lktA gene (StyI BamHI fragment from pAA345) was ligated to StyI BamHI-digested pAA342, yielding the plasmid pAA352. The structure of pAA352 is shown in Figure 2 and the nucleotide sequence and predicted amino acid sequence of *P. haemolytica* leukotoxin shown in Figure 3.

Example 2

Construction of LKT-antigen Fusions

Three representative LKT-antigen fusions were constructed as follows. Oligonucleotides containing sequences from the bovine rotavirus VP4, GnRH and SRIF genes were constructed on a Pharmacia Gene Assembler using standard phosphoramidite chemistry. The sequences of these oligonucleotides are shown in Figure 4. The oligonucleotides were annealed and ligated into the vector pAA352 (ATCC No. 68283, and described above), which had been digested with the restriction endonuclease BamHI. This vector contains the *P. haemolytica* leukotoxin gene. The ligated DNA was used to transform *E. coli* strain JM105 (in the case of SRIF) or MH3000 (for VP4 and GnRH). Transformants containing the oligonucleotide inserts were identified by restriction endonuclease mapping. Plasmid DNA from the *E. coli* MH3000 strains was then isolated and used to transform the strain JM105. The recombinant plasmids were designated pAA496 (LKT-SRIF, Figure 5), pAA502 (LKT-GnRH, Figure 7), and pAA501 (LKT-VP4, Figure 9). The nucleotide sequences of these three fusions are shown in Figures 6, 8 and 10, respectively.

Example 3

Purification of LKT-antigen Fusions

The recombinant LKT-antigen fusions from Example 2 were purified using the following procedure. For each fusion, five to ten colonies of the transformed *E. coli* strains were inoculated into 10 ml of TB broth supplemented with 100 micrograms/ml of ampicillin and incubated at 37°C for 6 hours on a G10 shaker, 220 rpm. Four ml of this culture was diluted into each of two baffled Fernbach flasks containing 400 ml of TB broth + ampicillin and incubated overnight as described above. Cells were harvested by centrifugation for 10 minutes at 4,000 rpm in polypropylene bottles, 500 ml volume, using a Sorvall GS3 rotor. The pellet was resuspended in an equal volume of TB broth containing ampicillin which had been prewarmed to 37°C (i.e., 2 x 400 ml), and the cells were incubated for 2 hours as described above.

3.2 ml of isopropyl-B,D-thiogalactopyranoside (IPTG, Gibco/BRL), 500 mM in water (final concentration = 4 mM), was added to each culture in order to induce synthesis of the recombinant fusion proteins. Cultures were incubated for two hours. Cells were harvested by centrifugation as described above, resuspended in 30 ml of 50 mM Tris-hydrochloride, 25% (w/v) sucrose, pH 8.0, and frozen at -70°C. The frozen cells were thawed at room temperature after 60 minutes at -70°C, and 5 ml of lysozyme (Sigma, 20 mg/ml in 250 mM Tris-HCl, pH 8.0) was added. The mixture was vortexed at high speed for 10 seconds and then placed on ice for 15 minutes. The cells were then added to 500 ml of lysis buffer in a 1000 ml beaker and mixed by stirring with a 2 ml pipette. The beaker containing the lysed cell suspension was placed on ice and sonicated for a total of 2.5 minutes (5-30 second bursts with 1 minute cooling between each) with a Braun sonicator, large probe, set at 100 watts power. Equal

volumes of the solution were placed in Teflon SS34
centrifuge tubes and centrifuged for 20 minutes at 10,000
rpm in a Sorvall SS34 rotor. The pellets were
resuspended in a total of 100 ml of sterile double
5 distilled water by vortexing at high speed, and the
centrifugation step repeated. Supernatants were
discarded and the pellets combined in 20 ml of 10 mM
Tris-HCl, 150 mM NaCl, pH 8.0 (Tris-buffered saline) and
the suspension frozen overnight at -20°C.

10 The recombinant suspension was thawed at room
temperature and added to 100 ml of 8 M Guanidine HCl
(Sigma) in Tris-buffered saline and mixed vigorously. A
magnetic stir bar was placed in the bottle and the
solubilized sample was mixed at room temperature for
15 30 minutes. The solution was transferred to a 2000 ml
Ehrlenmyer flask and 1200 ml of Tris-buffered saline was
quickly added. This mixture was stirred at room
temperature for an additional 2 hours. 500 ml aliquots
were placed in dialysis bags (Spectrum, 63.7 mm diameter,
20 6,000-8,000 MW cutoff, #132670, from Fisher scientific)
and these were placed in 4,000 ml beakers containing
3,500 ml of Tris-buffered saline + 0.5 M Guanidine HCl.
The beakers were placed in a 4°C room on a magnetic
stirrer overnight after which dialysis buffer was
25 replaced with Tris-buffered saline + 0.1 M Guanidine HCl
and dialysis continued for 12 hours. The buffer was then
replaced with Tris-buffered saline + 0.05 M Guanidine HCl
and dialysis continued overnight. The buffer was
replaced with Tris-buffered saline (no guanidine), and
30 dialysis continued for 12 hours. This was repeated three
more times. The final solution was poured into a 2000 ml
plastic roller bottle (Corning) and 13 ml of 100 mM PMSF
(in ethanol) was added to inhibit protease activity. The
solution was stored at -20°C in 100 ml aliquots.

5 To confirm that the fusion proteins had been isolated, aliquots of each preparation were diluted 20-fold in double distilled water, mixed with an equal volume of SDS-PAGE sample buffer, placed in a boiling water bath for five minutes and run through 12% polyacrylamide gels. Recombinant leukotoxin controls were also run. Western blots of the purification products were performed by reacting the LKT-SRIF preparation with swine anti-SRIF serum at a 1:500 dilution and the LKT-GnRH and LKT-VP4 preparations with mouse anti-VP4 serum at a 1:50 dilution. The only band visible in the LKT-SRIF western blot was that associated with the LKT-SRIF protein sample. No cross-reactivity with the leukotoxin was observed. Both the LKT-GnRH and LKT-VP4 proteins had similar apparent molecular weights, however, the anti-VP4 serum reacted only with the LKT-VP4 fusion protein.

20 All fusion proteins were expressed at high levels as inclusion bodies. The predicted molecular weights based on the DNA sequences of the three proteins (depicted in Figures 6, 8 and 10) were 101,366 (LKT-SRIF); 100,521 (LKT-GnRH); and 102,120 (LKT-VP4). The molecular weight of the recombinant leukotoxin molecule was 99,338. Both the SRIF and VP4 fusions were shown to react with monospecific antisera against the corresponding peptide.

Example 4

In Vivo Immunologic Activity of LKT-antigen Fusions

30 To test for enhanced immunogenicity of the LKT-antigen fusions as compared to the antigens alone, LKT-SRIF fusion protein was purified from *E. coli* cultures induced with IPTG, as described in Example 2. Aggregated protein was dissolved by treating with guanidine-HCl at a final concentration of three molar. The leukotoxin

concentration of this material was assayed using a standard quantitative leukotoxin specific ELISA. The assay utilized recombinant leukotoxin in 4 M guanidine-HCl (2 mg/ml) as a standard. Rabbit anti-leukotoxin antiserum was used as a detection and quantitation system.

A vaccine was formulated to a volume of 1 ml by mixing equal volumes of LKT-SRIF, diluted in Hanks Buffered Saline, and Emulsigen Plus (MVP Laboratories, Ralston, Nebraska). Four three month old lambs were immunized twice with 100 micrograms of fusion protein (containing an equivalent of approximately 1.4 micrograms of SRIF peptide). Blood samples were taken 10 days after the second injection and were analyzed for leukotoxin and SRIF specific antisera. All of the animals were found to have anti-leukotoxin titers of greater than 1 in 50,000, as determined by a leukotoxin specific ELISA. SRIF titers were assayed by a radioimmunoassay as described in Laarveld, B., et al., *Can. J. Anim. Sci.* (1986) 66:77-83. Two animals were found to have titers greater than 1 in 100.

To further test the ability of the LKT-SRIF chimeras to induce an anti-SRIF immunological response *in vivo*, and to compare this response to that produced by other SRIF conjugates, the following vaccination trial was performed. Three groups of 8 female pigs, approximately 8 weeks of age (35-50 kg) were used which were Specific Pathogen Free. The animals were maintained in a minimal disease facility and were vaccinated on days 0, 21 and 35 of the trial with the following formulations:

Group 1 -- placebo which was saline formulated in Emulsigen Plus adjuvant containing 15 mg DDA (Kodak) (2 ml);

Group 2 -- LKT-SRIF (250 μ g LKT, prepared as described above) formulated in the same adjuvant (2 ml);

Group 3 -- SRIF-avidin, biotinylated SRIF (10 μ g) and 2.5 μ g avidin, formulated in the same adjuvant (2 ml).

Blood samples were taken on days 0, 21 and 35, allowed to clot, centrifuged at 1500 g, and the serum removed. The serum antibody titers against SRIF were measured using the RIA procedure of Laarveld et al., *Can. J. Anim. Sci.* (1986) 66:77-83.

7 of the 8 animals immunized with the LKT-SRIF formulation produced significant titers against SRIF (>1:700) whereas only 2 of 8 animals immunized with the SRIF-Avidin responded with serum titers of >700.

This example demonstrates that leukotoxin chimeric molecules are highly immunogenic. It has been reported by Laarveld, et al., *Can. J. Animal Sci.* (1986) 66:77, that repeated immunization with greater than 100 micrograms of SRIF peptide conjugated to an ovalbumin carrier was necessary to evoke an immune reaction.

Example 5

In Vivo Immunologic Activity of LKT-GnRH Fusions

To test for the ability of LKT-GnRH to induce an anti GnRH immunological response *in vivo*, and to compare this response to other GnRH carrier conjugates, the following vaccination trial was performed. Three groups of 8 male pigs, approximately 8 weeks of age (35-50 kg) were used which were Specific Pathogen Free. The animals were maintained in a minimal disease facility and were vaccinated on days 0 and 21 of the trial with the following formulations:

Group 1 -- placebo which consisted of saline formulated in Emulsigen Plus adjuvant containing 15 mg of DDA (2 ml);

-41-

Group 2 -- LKT-GnRH (250 μ g LKT, prepared as described in the previous examples) formulated in the same adjuvant (2 ml);

Group 3 -- VP6-GnRH, 0.5 μ g VP6 and 5 μ g GnRH, formulated in the same adjuvant (2 ml). The VP6 preparation was made as described in U.S. Patent No. 5,071,651, using the binding peptide described therein.

Blood samples were taken on days 0, 21 and 35, allowed to clot, centrifuged at 1500 g, and the serum removed. The serum antibody titers against GnRH were measured using the RIA procedure of Silversides et al., *J. Reprod. Immunol.* (1985) 7:171-184.

The results of this trial indicated that only those animals immunized with the LKT-GnRH formulation produced significant titers against GnRH (titers >1:70). Neither the placebo nor the VP6-GnRH groups produced anti-GnRH titers. Previously, multiple vaccinations with doses of GnRH of more than 100 μ g, conjugated to other carrier proteins, were required to induce anti-hormone titers.

Thus, chimeric proteins including leukotoxin fused to a selected antigen, have been disclosed. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

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7. The carrier system of claim 6 wherein said chimeric protein comprises the amino acid sequence depicted in Figure 8, or an amino acid sequence substantially homologous and functionally equivalent thereto.

8. The carrier system of claim 1 wherein said selected antigen is bovine rotavirus VP4, or an epitope thereof.

5 9. The carrier system of claim 8 wherein said chimeric protein comprises the amino acid sequence depicted in Figure 10, or an amino acid sequence substantially homologous and functionally equivalent thereto.

10 10. A vaccine composition comprising the chimeric protein of claim 1 and a pharmaceutically acceptable vehicle.

15 11. A vaccine composition comprising the chimeric protein of claim 4 and a pharmaceutically acceptable vehicle.

20 12. A vaccine composition comprising the chimeric protein of claim 6 and a pharmaceutically acceptable vehicle.

25 13. A vaccine composition comprising the chimeric protein of claim 8 and a pharmaceutically acceptable vehicle.

30 14. A method for presenting a selected antigen to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 10.

35 15. A method for presenting a selected antigen to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 11.

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23. The DNA construct of claim 18 wherein said second nucleotide sequence encodes bovine rotavirus VP4, or an epitope thereof.

5 24. The DNA construct of claim 23 comprising the nucleotide sequence depicted in Figure 10 or a nucleotide sequence substantially homologous and functionally equivalent thereto.

10 25. An expression cassette comprised of:
 (a) the DNA construct of claim 18; and
 (b) control sequences that direct the transcription of said construct whereby said construct can be transcribed and translated in a host cell.

15 26. An expression cassette comprised of:
 (a) the DNA construct of claim 19; and
 (b) control sequences that direct the transcription said construct whereby said construct can
20 be transcribed and translated in a host cell.

 27. An expression cassette comprised of:
 (a) the DNA construct of claim 21; and
 (b) control sequences that direct the
25 transcription said construct whereby said construct can be transcribed and translated in a host cell.

 28. An expression cassette comprised of:
 (a) the DNA construct of claim 23; and
30 (b) control sequences that direct the transcription said construct whereby said construct can be transcribed and translated in a host cell.

 29. A host cell stably transformed with the
35 expression cassette of claim 25.

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

[illegible][illegible][illegible][illegible][illegible]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

[illegible][illegible]

-47-

(a) providing a population of host cells
according to claim 32; and

(b) growing said population of cells under
conditions whereby the polypeptide encoded by said
5 expression cassette is expressed.

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ENHANCED IMMUNOGENICITY USING LEUKOTOXIN CHIMERAS

Abstract of the Disclosure

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New immunological carrier systems, DNA encoding
the same, and the use of these systems, are disclosed.
The carrier systems include chimeric proteins which
comprise a leukotoxin polypeptide fused to a selected
10 antigen. The leukotoxin functions to increase the
immunogenicity of the antigen fused thereto.

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VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS

(37 CFR 1.9(f) & 1.27(d))--NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF NONPROFIT ORGANIZATION: University of Saskatchewan

ADDRESS OF NONPROFIT ORGANIZATION: Saskatoon, Saskatchewan S7N 0W0

TYPE OF NONPROFIT ORGANIZATION:

- ☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
☐ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE 26 USC 501(a) and 501(c)(3)
☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF U.S.A.
 (Name of State: _____)
 (Citation of Statute: _____)
☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE
 26 USC 501(a) and 501(c)(3) IF LOCATED IN THE UNITED STATES OF AMERICA
☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE
 OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN U.S.A.
 (Name of State: _____)
 (Citation of Statute: _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention entitled: ENHANCED IMMUNOGENICITY USING LEUKOTOXIN CHIMERAS described in:

- ☒ the specification filed herewith.
☐ application serial no. *, filed *.
☐ patent no. *, issued *.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization regarding the above-identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights in the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

* NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME: *

ADDRESS: *

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

NAME: *

ADDRESS: *

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

SIGNATURE

NAME OF PERSON SIGNING:

TITLE IN ORGANIZATION OF PERSON SIGNING:

ADDRESS OF PERSON SIGNING: University of Saskatchewan, S7N 0W0

DATE:

Oct 2/92

00976566-112197

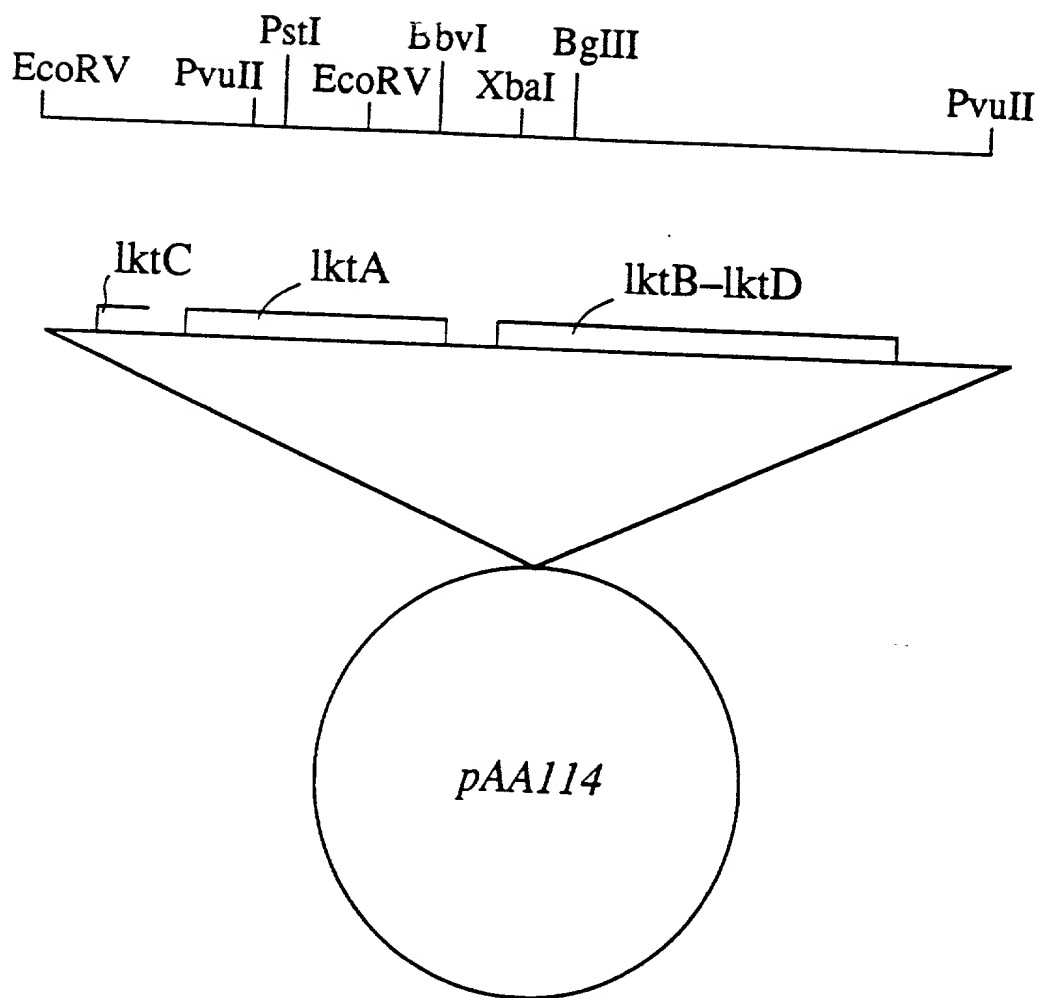


Figure 1

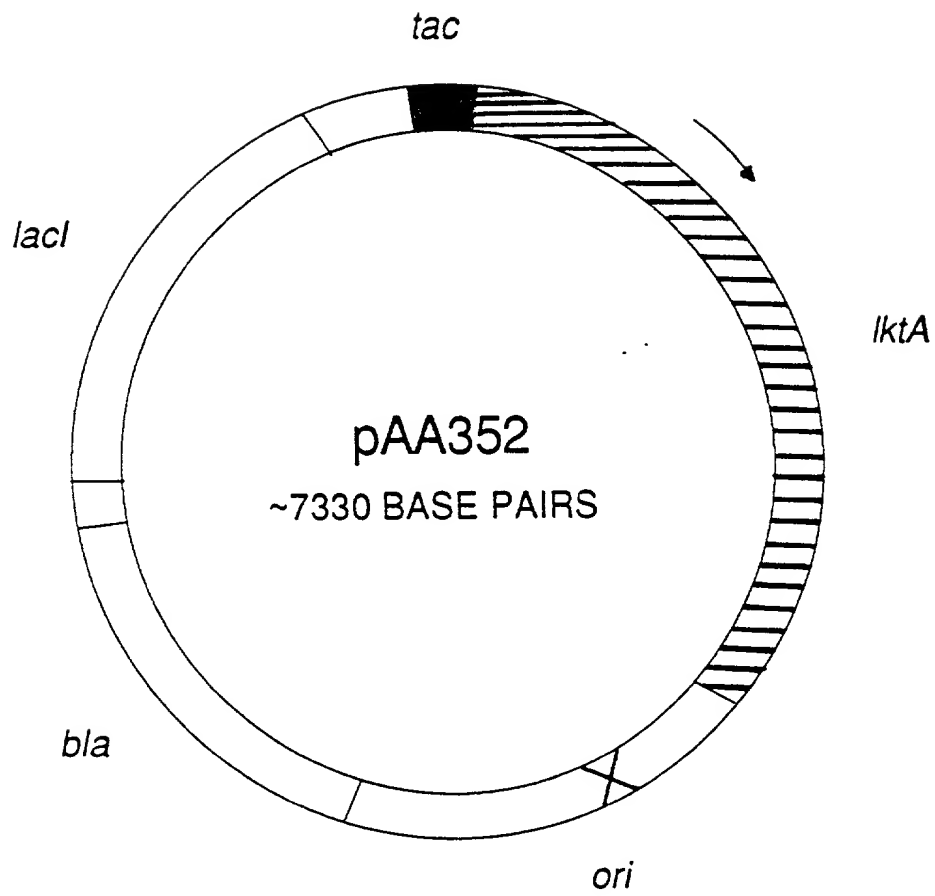


Figure 2

[illegible]

	100	110	120	130	140	150	160	170	180																					
CAA	GGT	AAT	GGT	TTA	CAG	6AT	TTA	GTC	AAA	6CG	6CC	GAA	6AG	TTG	6GG	ATT	6AG	6TA	CAA	AGA	6AA	6AA	6CG	AAT	AAT	ATT	6CA	ACA	6CT	
GTT	CCA	TTA	CCA	AAT	GTC	CTA	AAT	CAG	TTT	CGC	CGG	CTT	CTC	AAC	CCC	TAA	CTC	CAT	6TT	TCT	TCT	CTT	CTT	6CG	TTA	TTA	TAA	6GT	TGT	CGA
Gln	Gly	Asn	Gly	Leu	Gln	Asp	Leu	Val	Lys	Ala	Ala	Glu	Glu	Leu	Gly	Ile	Glu	Val	Gln	Arg	Glu	Glu	Arg	Asn	Asn	Ile	Ala	Thr	Ala	

[illegible]

Figure 3-2

280	290	300	310	320	330	340	350	360
#	#	#	#	#	#	#	#	#
AAA ACT AAA GCA GGC CAA TTA GGT ICT GGC GAA AGC ATT GTA CAA AAT GCA AAT AAA GCC AAA ACT GTA TTA ICT GGC ATT CAA ICT	TTT TGA TTT CGT CCG GTT CGT AAT CCA AGA CCG CTT TCG TAA CAT GTT TTA CGT TTA TTT CCG TTT TGA CAT AAT AGA CCG TAA GTT AGA	Lys Thr Lys Ala Gln Ala Leu Gln Ser Ala Gln Ser Ile Val Gln Asn Ala Asn Lys Ala Lys Thr Val Leu Ser Gln Ile Gln Ser>	----- RECOMBINANT LEUKOTOXIN PEPTIDE ----->					
370	380	390	400	410	420	430	440	450
#	#	#	#	#	#	#	#	#
ATT TTA GGC TCA GTA TTG GCT GGA ATG GAT TTA GAT GAG GCC TTA CAG AAT AAC AGC AAC CAA CAT GCT CTT GCT AAA GCT GGC TTG GAG	TAA AAT CCG AGT CAT AAC CGA CCT TAC CTA AAT CTA CTC CCG AAT GTC TTA TTG TCG TTG GTT GTA CGA GAA CCA TTT CGA CCG AAC CTC	Ile Leu Gln Ser Val Leu Ala Gln Met Asp Leu Asp Gln Ala Leu Gln Asn Ser Asn Gln His Ala Leu Ala Lys Ala Gln Leu Gln>	----- RECOMBINANT LEUKOTOXIN PEPTIDE ----->					
460	470	480	490	500	510	520	530	540
#	#	#	#	#	#	#	#	#
CTA ACA AAT TCA TTA ATT GAA AAT ATT GCT AAT TCA GTA AAA ACA CTT GAC GAA TTT GGT GAG CAA ATT AGT CAA TTT GGT TCA AAA CTA	GAT TGT TTA AGT AAT TAA CTT TTA TAA CGA TTA AGT CAT TTT TGT GAA CTG CTT AAA CCA CTC GTC TAA TCA GTC AAA CCA AGT TTT GAT	Leu Thr Asn Ser Leu Ile Gln Asn Ile Ala Asn Ser Val Lys Thr Leu Asp Gln Phe Gln Ile Ser Gln Phe Gln Ser Lys Leu>	----- RECOMBINANT LEUKOTOXIN PEPTIDE ----->					
550	560	570	580	590	600	610	620	630
#	#	#	#	#	#	#	#	#
CAA AAT ATC AAA GGC TTA GGC ACT TTA GGA GAC AAA CTC AAA AAT ATC GGT GGA CTT GAT AAA GCT GGC CTT GGT TTA GAT GTT ATC TCA	GTT TTA TAG TTT CCG AAT CCC TGA AAT CCT CTG TTT GAG TTT TTA TAG CCA CCT GAA CTA TTT CGA CCG GAA CCA AAT CTA CAA TAG AGT	Gln Asn Ile Lys Gln Leu Gln Thr Leu Gln Asp Lys Leu Lys Asn Ile Gln Gln Ser Lys Ala Gln Leu Gln Asp Val Ile Ser>	----- RECOMBINANT LEUKOTOXIN PEPTIDE ----->					

Figure 3-4

910	920	930	940	950	960	970	980	990
†	†	†	†	†	†	†	†	†
GAG AGT TAT GCC GAA CGC TTT AAA AAA TTA GGC TAT GAC GGA GAT AAT TTA TTA GCA GAA TAT CAG CCG GGA ACA GGG ACT ATT GAT GCA								
CTC TCA ATA CGG CTT GCG AAA TTT TTT AAT CCG ATA CTG CCT CTA TTA AAT AAT CGT CTT ATA GTC GCC CCT TGT CCC TGA TAA CTA CGT								
Glu Ser Tyr Ala Glu Arg Phe Lys Lys Leu Gly Tyr Asp Gly Asn Leu Leu Ala Glu Tyr Gln Arg Gly Thr Gln Thr Ile Asp Ala>								
RECOMBINANT LEUKOTOXIN PEPTIDE								
1000	1010	1020	1030	1040	1050	1060	1070	1080
†	†	†	†	†	†	†	†	†
TCG GTT ACT GCA ATT AAT ACC GCA TTG GCC GCT ATT GCT GGT GGT GGT TCT GCT GCT GCA GCC GGC TCG GTT ATT GCT TCA CCG ATT GCC								
AGC CAA TGA CGT TAA TTA TGG CGT AAC CCG CGA TAA CGA CCA CCA CAC AGA CGA CGA CGT CCG CCG AGC CAA TAA CGA AGT GGC TAA CCG								
Ser Val Thr Ala Ile Asn Thr Ala Leu Ala Ala Ile Ala Gly Val Ser Ala Ala Ala Gln Ser Val Ile Ala Ser Pro Ile Ala>								
RECOMBINANT LEUKOTOXIN PEPTIDE								
1090	1100	1110	1120	1130	1140	1150	1160	1170
†	†	†	†	†	†	†	†	†
TTA TTA GTA TCT GGG ATT ACC GGT GTA ATT TCT ACG ATT CTG CAA TAT TCT AAA CAA GCA ATG TTT GAG CAC GAT GCA AAT AAA ATT CAT								
AAT AAT CAT AGA CCC TAA TGG CCA CAT TAA AGA TGC TAA GAC GTT ATA AGA TTT GTT CGT TAC AAA CTC GTG CAA CGT TTA TTT TAA GTA								
Leu Leu Val Ser Gly Ile Thr Gly Val Ile Ser Thr Ile Leu Gln Tyr Ser Lys Gln Ala Met Phe Glu His Val Ala Asn Lys Ile His>								
RECOMBINANT LEUKOTOXIN PEPTIDE								
1180	1190	1200	1210	1220	1230	1240	1250	1260
†	†	†	†	†	†	†	†	†
AAC AAA ATT GTA GAA TGG GAA AAA AAT AAT CAC GGT AAG AAC TAC TTT GAA AAT GGT TAC GAT GCC CGT TAT CTT GCG AAT TTA CAA GAT								
TTG TTT TAA CAT CTT ACC CTT TTT TTA TTA GTG CCA TTC TTG ATG AAA CTT TTA CCA ATG CTA CCG GCA ATA GAA CCG TTA AAT GTT CTA								
Asn Lys Ile Val Glu Trp Glu Lys Asn His Gly Lys Asn Tyr Phe Glu Asn Gly Tyr Asp Ala Arg Tyr Leu Ala Asn Leu Gln Asp>								
RECOMBINANT LEUKOTOXIN PEPTIDE								

Figure 3-5

1270	1280	1290	1300	1310	1320	1330	1340	1350
#	#	#	#	#	#	#	#	#
AAT ATG AAA TTC TTA CTG AAC TTA AAC AAA GAG TTA CAG GCA GGT GTC ATC GCT ATT ACT CAG CAG CAA TGG GAT AAC AAC ATT GGT	TTA TAC TTT AAG AAT GAC TTG AAT TTG TTT CTC AAT GTC CGT CTT GCA CAG TAG CGA TAA TGA GTC GTC GTT ACC CTA TTG TTG TAA CCA	Asn Met Lys Phe Leu Leu Asn Leu Asn Lys Glu Leu Glu Ala Glu Arg Val Ile Ala Ile Thr Glu Glu Trp Asp Asn Asn Ile Gly>	RECOMBINANT LEUKOTOXIN PEPTIDE					
1360	1370	1380	1390	1400	1410	1420	1430	1440
#	#	#	#	#	#	#	#	#
GAT TTA GCT GGT ATT AGC CGT TTA GGT GAA AAA GTC CTT AGT GGT AAA GCC TAT GTG GAT GCG TTT GAA GAA GGC AAA CAC ATT AAA GCC	CTA AAT CGA CCA TAA TCG GCA AAT CCA CTT TTT CAG GAA TCA CCA TTT CGG ATA CAC CTA CCG AAA CTT CTT CCG TTT GTG TAA TTT CGG	Asp Leu Ala Gly Ile Ser Arg Leu Leu Gly Glu Lys Val Leu Ser Gly Lys Ala Tyr Val Asp Ala Phe Glu Glu Gly Lys His Ile Lys Ala>	RECOMBINANT LEUKOTOXIN PEPTIDE					
1450	1460	1470	1480	1490	1500	1510	1520	1530
#	#	#	#	#	#	#	#	#
GAT AAA TTA GTA CAG TTG GAT TCG GCA AAC GGT ATT ATT GAT GTG AGT AAT TCG GGT AAA GCG AAA ACT CAG CAT ATC TTA TTC AGA ACG	CTA TTT AAT CAT GTC AAC CTA AGC CGT TTG CCA TAA TAA CTA CAC TCA TTA AGC CCA TTT CCG TTT TGA GTC GTA TAG AAT AAG TCT TGC	Asp Lys Leu Val Glu Leu Asp Ser Ala Asn Gly Ile Ile Asp Val Ser Asn Ser Gly Lys Ala Lys Thr Glu His Ile Leu Phe Arg Thr>	RECOMBINANT LEUKOTOXIN PEPTIDE					

Figure 3-8

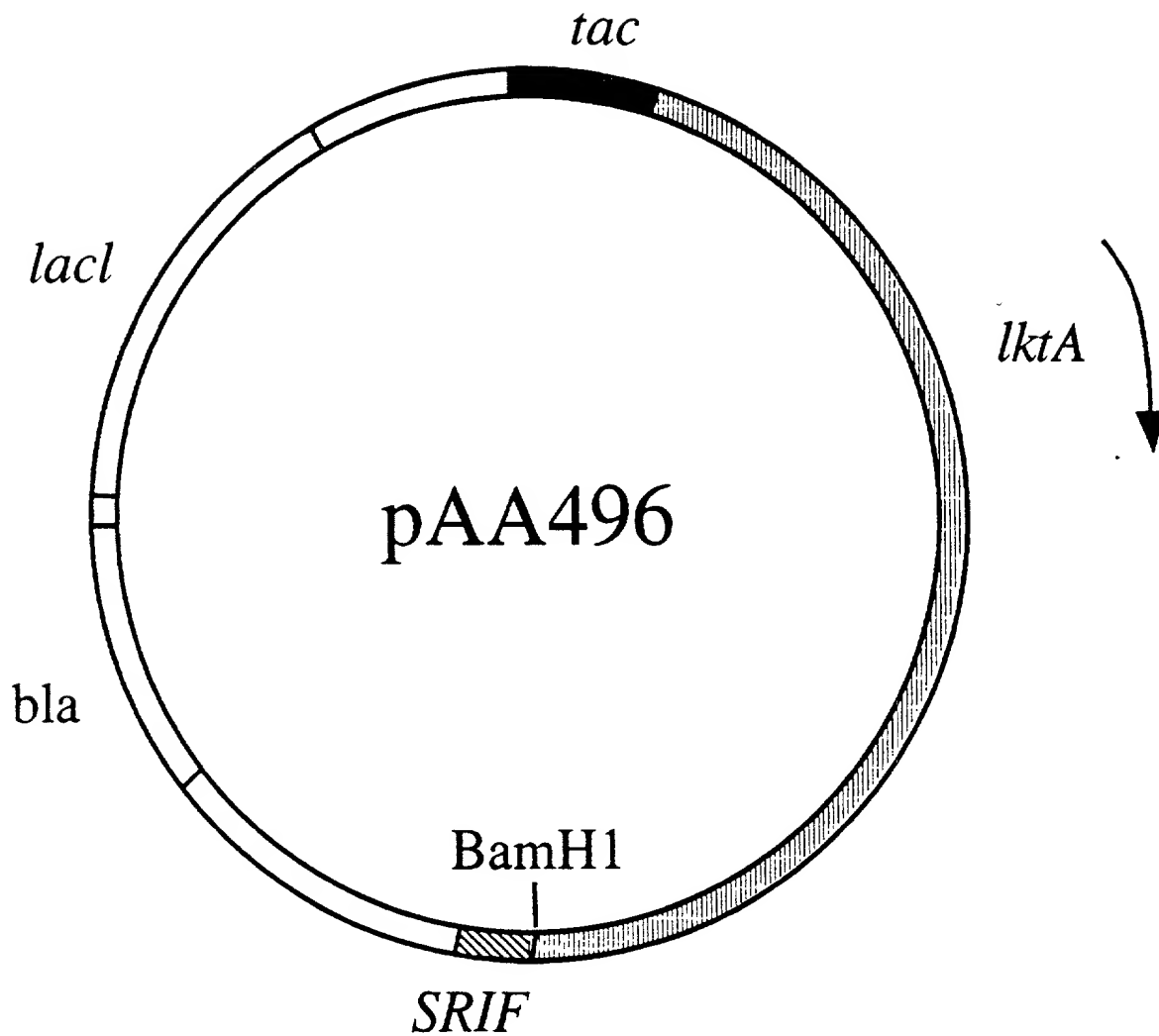
2170	2180	2190	2200	2210	2220	2230	2240	2250
* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *
GAC C6C TTA TTT GGT 6GT AAA GGC GAT GAT ATT CTC GAT GGT GGA AAT 6GT GAT GAT TTT ATC GAT GGC 6GT AAA GGC AAC GAC CTA TTA	CTG GCG AAT AAA CCA CCA TTT CCG CTA CTA TAA GAG CTA CCA CCT TTA CCA CTA CTA AAA TAG CTA CCG CCA TTT CCG TTG CTG GAT AAT	Asp Arg Leu Phe Gly Gly Lys Gly Asp Ile Leu Asp Asp Gly Asp Asp Phe Ile Asp Gly Gly Lys Gly Asn Asp Leu Leu>	RECOMBINANT LEUKOTOXIN PEPTIDE<----->					
2260	2270	2280	2290	2300	2310	2320	2330	2340
* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *
CAC GGT 6GC AAG GGC GAT GAT ATT TTC GTT CAC CGT AAA GGC GAT GGT AAT GAT GAT ATT ACC GAT TCT GAC GGC AAT GAT AAA TTA TCA	GTG CCA CCG TTC CCG CTA CTA TAA AAG CAA GIG GCA TTT CCG CTA CCA TTA CTA TAA TGG CTA AGA CIG CCG TTA CTA TTT AAT AGT	His Gly Gly Lys Gly Asp Ile Phe Val His Arg Lys Gly Asp 6Ly Asn Asp Ile Ile Thr Asp Ser Asp 6Ly Asn Asp Lys Leu Ser>	RECOMBINANT LEUKOTOXIN PEPTIDE<----->					
2350	2360	2370	2380	2390	2400	2410	2420	2430
* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *
TTC TCT GAT TCG AAC TTA AAA GAT TTA ACA TTT GAA AAA GTT AAA CAT AAT CTT GTC ATC ACG AAT AGC AAA AAA GAG AAA GTG ACC ATT	AAG AGA CTA AGC TTG AAT TTT CTA AAT TGT AAA CTT TTT CAA TTT GTA TTA GAA CAG TAG TGC TTA TCG TTT TTT TTT CTC TTT CAC TGG TAA	Phe Ser Asp Ser Asn Leu Lys Asp Leu Thr Phe Thr Phe Glu Lys Val Lys Lys His Asn Leu Val Ile Thr Asn Ser Lys Lys Glu Lys Val Thr Ile>	RECOMBINANT LEUKOTOXIN PEPTIDE<----->					
2440	2450	2460	2470	2480	2490	2500	2510	2520
* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *
CAA AAC TGG TTC CGA GAG GCT GAT TTT GCT AAA GAA GAA GTG CCT AAT TAT AAA GCA ACT AAA GAT GAG AAA ATC GAA GAA ATC ATC GGT CAA	6TT TTG ACC AAG GCT CTC CGA CTA AAA CGA TTT CTT CAC GGA TTA ATA TTT CGT TGA TTT CTA CTC TTT TAG CTT TAG TAG CCA GTT	Gln Asn Trp Phe Arg Glu Ala Asp Phe Ala Lys Glu Val Pro Asn Tyr Lys Ala Thr Lys Asp Glu Lys Ile Glu Glu Ile Ile Glu Gln>	RECOMBINANT LEUKOTOXIN PEPTIDE<----->					

SRIF-1: 5'-GATCAGCTCTTCTGCGGCTGCAGAAACTTCTCTGGAAGAACCTTCACCAAGCTGCTAGG-3'
 SRIF-2: 3'-GTGAGAGACAGCGCGGAGCGTTTGTGAAGAACCTTTTGGAGTGGTCCGACGATCCCTAG-5'

GNRH-1: 5'-GATCTCAGCATTTGGAGCTAAGGCTGCGGCTGCTGCTAAG-3'
 GNRH-2: 3'-AGTGTAAACCTCGATGCGGAGCGGCGGAGTTCCTAG-5'

VP4-1: 5'-GATCTTGCACATTTGTGCTGTGAGCATTTGTGAGCGGCAACATTTGTGTACACCGCGGCAACTAACCAAGACATTTGTGTAG-3'
 VP4-2: 3'-AACGTTGTAAACACGGGACACTCGTAAACACTCGCGGTTGTAAACACATGTGGGCGGCGGTTGAATTTGGTTCGTAAACACATCTCTAG-5'

Figure 4



tac = hybrid *trp::lac* promoter from *E. coli*
bla = beta lactamase gene (ampicillin resistance)
lktA = *Pasteurella haemolytica* structural gene
SRIF = SRIF structural gene
lacI = *E. coli lac* operon repressor

Figure 5

```

      10      20      30      40
      *      *      *      *      *      *      *      *
ATG GCT ACT GTT ATA GAT CTA AGC TTC CCA AAA ACT GGG GCA AAA AAA
TAC CGA TGA CAA TAT CTA GAT TCG AAG GGT TTT TGA CCC CGT TTT TTT
Met Ala Thr Val Ile Asp Leu Ser Phe Pro Lys Thr Gly Ala Lys Lys>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

50      60      70      80      90
      *      *      *      *      *      *      *      *
ATT ATC CTC TAT ATT CCC CAA AAT TAC CAA TAT GAT ACT GAA CAA GGT
TAA TAG GAG ATA TAA GGG GTT TTA ATG GTT ATA CTA TGA CTT GTT CCA
Ile Ile Leu Tyr Ile Pro Gln Asn Tyr Gln Tyr Asp Thr Glu Gln Gly>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

100      110      120      130      140
      *      *      *      *      *      *      *      *
AAT GGT TTA CAG GAT TTA GTC AAA GCG GCC GAA GAG TTG GGG ATT GAG
TTA CCA AAT GTC CTA AAT CAG TTT CGC CGG CTT CTC AAC CCC TAA CTC
Asn Gly Leu Gln Asp Leu Val Lys Ala Ala Glu Glu Leu Gly Ile Glu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

150      160      170      180      190
      *      *      *      *      *      *      *      *
GTA CAA AGA GAA GAA CGC AAT AAT ATT GCA ACA GCT CAA ACC AGT TTA
CAT GTT TCT CTT CTT GCG TTA TTA TAA CGT TGT CGA GTT TGG TCA AAT
Val Gln Arg Glu Glu Arg Asn Asn Ile Ala Thr Ala Gln Thr Ser Leu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

200      210      220      230      240
      *      *      *      *      *      *      *      *
GGC ACG ATT CAA ACC GCT ATT GGC TTA ACT GAG CGT GGC ATT GTG TTA
CCG TGC TAA GTT TGG CGA TAA CCG AAT TGA CTC GCA CCG TAA CAC AAT
Gly Thr Ile Gln Thr Ala Ile Gly Leu Thr Glu Arg Gly Ile Val Leu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

```

Figure 6-1

250 260 270 280
 * * * * * * *
 TCC GCT CCA CAA ATT GAT AAA TTG CTA CAG AAA ACT AAA GCA GGC CAA
 AGG CGA GGT GTT TAA CTA TTT AAC GAT GTC TTT TGA TTT CGT CCG GTT
 Ser Ala Pro Gln Ile Asp Lys Leu Leu Gln Lys Thr Lys Ala Gly Gln>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

290 300 310 320 330
 * * * * * * *
 GCA TTA GGT TCT GCC GAA AGC ATT GTA CAA AAT GCA AAT AAA GCC AAA
 CGT AAT CCA AGA CCG CTT TCG TAA CAT GTT TTA CGT TTA TTT CGG TTT
 Ala Leu Gly Ser Ala Glu Ser Ile Val Gln Asn Ala Asn Lys Ala Lys>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

340 350 360 370 380
 * * * * * * *
 ACT GTA TTA TCT GGC ATT CAA TCT ATT TTA GGC TCA GTA TTG GCT GGA
 TGA CAT AAT AGA CCG TAA GTT AGA TAA AAT CCG AGT CAT AAC CGA CCT
 Thr Val Leu Ser Gly Ile Gln Ser Ile Leu Gly Ser Val Leu Ala Gly>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

390 400 410 420 430
 * * * * * * *
 ATG GAT TTA GAT GAG GCC TTA CAG AAT AAC AGC AAC CAA CAT GCT CTT
 TAC CTA AAT CTA CTC CGG AAT GTC TTA TTG TCG TTG GTT GTA CGA GAA
 Met Asp Leu Asp Glu Ala Leu Gln Asn Asn Ser Asn Gln His Ala Leu>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

440 450 460 470 480
 * * * * * * *
 GCT AAA GCT GGC TTG GAG CTA ACA AAT TCA TTA ATT GAA AAT ATT GCT
 CGA TTT CGA CCG AAC CTC GAT TGT TTA AGT AAT TAA CTT TTA TAA CGA
 Ala Lys Ala Gly Leu Glu Leu Thr Asn Ser Leu Ile Glu Asn Ile Ala>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

490 500 510 520
 * * * * * * *
 AAT TCA GTA AAA ACA CTT GAC GAA TTT GGT GAG CAA ATT AGT CAA TTT
 TTA AGT CAT TTT TGT GAA CTG CTT AAA CCA CTC GTT TAA TCA GTT AAA
 Asn Ser Val Lys Thr Leu Asp Glu Phe Gly Glu Gln Ile Ser Gln Phe>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

Figure 6-2

530 540 550 560 570
 * * * * *
 GGT TCA AAA CTA CAA AAT ATC AAA GGC TTA GGG ACT TTA GGA GAC AAA
 CCA AGT TTT GAT GTT TTA TAG TTT CCG AAT CCC TGA AAT CCT CTG TTT
 Gly Ser Lys Leu Gln Asn Ile Lys Gly Leu Gly Thr Leu Gly Asp Lys>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

580 590 600 610 620
 * * * * *
 CTC AAA AAT ATC GGT GGA CTT GAT AAA GCT GGC CTT GGT TTA GAT GTT
 GAG TTT TTA TAG CCA CCT GAA CTA TTT CGA CCG GAA CCA AAT CTA CAA
 Leu Lys Asn Ile Gly Gly Leu Asp Lys Ala Gly Leu Gly Leu Asp Val>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

630 640 650 660 670
 * * * * *
 ATC TCA GGG CTA TTA TCG GGC GCA ACA GCT GCA CTT GTA CTT GCA GAT
 TAG AGT CCC GAT AAT AGC CCG CGT TGT CGA CGT GAA CAT GAA CGT CTA
 Ile Ser Gly Leu Leu Ser Gly Ala Thr Ala Ala Leu Val Leu Ala Asp>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

680 690 700 710 720
 * * * * *
 AAA AAT GCT TCA ACA GCT AAA AAA GTG GGT GCG GGT TTT GAA TTG GCA
 TTT TTA CGA AGT TGT CGA TTT TTT CAC CCA CGC CCA AAA CTT AAC CGT
 Lys Asn Ala Ser Thr Ala Lys Lys Val Gly Ala Gly Phe Glu Leu Ala>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

730 740 750 760
 * * * *
 AAC CAA GTT GTT GGT AAT ATT ACC AAA GCC GTT TCT TCT TAC ATT TTA
 TTG GTT CAA CAA CCA TTA TAA TGG TTT CGG CAA AGA AGA TAA AAT
 Asn Gln Val Val Gly Asn Ile Thr Lys Ala Val Ser Ser Tyr Ile Leu>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

770 780 790 800 810
 * * * * *
 GCC CAA CGT GTT GCA GCA GGT TTA TCT TCA ACT GGG CCT GTG GCT GCT
 CGG GTT GCA CAA CGT CGT CCA AAT AGA AGT TGA CCC GGA CAC CGA CGA
 Ala Gln Arg Val Ala Ala Gly Leu Ser Ser Thr Gly Pro Val Ala Ala>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

820 830 840 850 860
 * * * * *
 TTA ATT GCT TCT ACT GTT TCT CTT GCG ATT AGC CCA TTA GCA TTT GCC
 AAT TAA CGA AGA TGA CAA AGA GAA CGC TAA TCG GGT AAT CGT AAA CGG
 Leu Ile Ala Ser Thr Val Ser Leu Ala Ile Ser Pro Leu Ala Phe Ala>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

Figure 6-3

870 880 890 900 910
 * * * * * * *
 GGT ATT GCC GAT AAA TTT AAT CAT GCA AAA AGT TTA GAG AGT TAT GCC
 CCA TAA CGG CTA TTT AAA TTA GTA CGT TTT TCA AAT CTC TCA ATA CGG
 Gly Ile Ala Asp Lys Phe Asn His Ala Lys Ser Leu Glu Ser Tyr Ala>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

920 930 940 950 960
 * * * * * * *
 GAA CGC TTT AAA AAA TTA GGC TAT GAC GGA GAT AAT TTA TTA GCA GAA
 CTT GCG AAA TTT TTT AAT CCG ATA CTG CCT CTA TTA AAT AAT CGT CTT
 Glu Arg Phe Lys Lys Leu Gly Tyr Asp Gly Asp Asn Leu Leu Ala Glu>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

970 980 990 1000
 * * * * * * *
 TAT CAG CGG GGA ACA GGG ACT ATT GAT GCA TCG GTT ACT GCA ATT AAT
 ATA GTC GCC CCT TGT CCC TGA TAA CTA CGT AGC CAA TGA CGT TAA TTA
 Tyr Gln Arg Gly Thr Gly Thr Ile Asp Ala Ser Val Thr Ala Ile Asn>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1010 1020 1030 1040 1050
 * * * * * * *
 ACC GCA TTG GCC GCT ATT GCT GGT GGT GTG TCT GCT GCT GCA GCC GGC
 TGG CGT AAC CGG CGA TAA CGA CCA CCA CAC AGA CGA CGA CGT CGG CCG
 Thr Ala Leu Ala Ala Ile Ala Gly Gly Val Ser Ala Ala Ala Gly>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1060 1070 1080 1090 1100
 * * * * * * *
 TCG GTT ATT GCT TCA CCG ATT GCC TTA TTA GTA TCT GGG ATT ACC GGT
 AGC CAA TAA CGA AGT GGC TAA CGG AAT AAT CAT AGA CCC TAA TGG CCA
 Ser Val Ile Ala Ser Pro Ile Ala Leu Leu Val Ser Gly Ile Thr Gly>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1110 1120 1130 1140 1150
 * * * * * * *
 GTA ATT TCT ACG ATT CTG CAA TAT TCT AAA CAA GCA ATG TTT GAG CAC
 CAT TAA AGA TGC TAA GAC GTT ATA AGA TTT GTT CGT TAC AAA CTC GTG
 Val Ile Ser Thr Ile Leu Gln Tyr Ser Lys Gln Ala Met Phe Glu His>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

Figure 6-4

1160 1170 1180 1190 1200
 * * * * * * * *
 GTT GCA AAT AAA ATT CAT AAC AAA ATT GTA GAA TGG GAA AAA AAT AAT
 CAA CGT TTA TTT TAA GTA TTG TTT TAA CAT CTT ACC CTT TTT TTA TTA
 Val Ala Asn Lys Ile His Asn Lys Ile Val Glu Trp Glu Lys Asn Asn>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

1210 1220 1230 1240
 * * * * * * *
 CAC GGT AAG AAC TAC TTT GAA AAT GGT TAC GAT GCC CGT TAT CTT GCG
 GTG CCA TTC TTG ATG AAA CTT TTA CCA ATG CTA CGG GCA ATA GAA CGC
 His Gly Lys Asn Tyr Phe Glu Asn Gly Tyr Asp Ala Arg Tyr Leu Ala>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

1250 1260 1270 1280 1290
 * * * * * * * *
 AAT TTA CAA GAT AAT ATG AAA TTC TTA CTG AAC TTA AAC AAA GAG TTA
 TTA AAT GTT CTA TTA TAC TTT AAG AAT GAC TTG AAT TTG TTT CTC AAT
 Asn Leu Gln Asp Asn Met Lys Phe Leu Leu Asn Leu Asn Lys Glu Leu>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

1300 1310 1320 1330 1340
 * * * * * * * *
 CAG GCA GAA CGT GTC ATC GCT ATT ACT CAG CAG CAA TGG GAT AAC AAC
 GTC CGT CTT GCA CAG TAG CGA TAA TGA GTC GTC GTT ACC CTA TTG TTG
 Gln Ala Glu Arg Val Ile Ala Ile Thr Gln Gln Gln Trp Asp Asn Asn>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

1350 1360 1370 1380 1390
 * * * * * * * *
 ATT GGT GAT TTA GCT GGT ATT AGC CGT TTA GGT GAA AAA GTC CTT AGT
 TAA CCA CTA AAT CGA CCA TAA TCG GCA AAT CCA CTT TTT CAG GAA TCA
 Ile Gly Asp Leu Ala Gly Ile Ser Arg Leu Gly Glu Lys Val Leu Ser>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

1400 1410 1420 1430 1440
 * * * * * * * *
 GGT AAA GCC TAT GTG GAT GCG TTT GAA GAA GGC AAA CAC ATT AAA GCC
 CCA TTT CGG ATA CAC CTA CGC AAA CTT CTT CCG TTT GTG TAA TTT CGG
 Gly Lys Ala Tyr Val Asp Ala Phe Glu Glu Gly Lys His Ile Lys Ala>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

1450 1460 1470 1480
 * * * * * * *
 GAT AAA TTA GTA CAG TTG GAT TCG GCA AAC GGT ATT ATT GAT GTG AGT
 CTA TTT AAT CAT GTC AAC CTA AGC CGT TTG CCA TAA TAA CTA CAC TCA
 Asp Lys Leu Val Gln Leu Asp Ser Ala Asn Gly Ile Ile Asp Val Ser>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

Figure 6-5

08976566.4349

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1490          1500          1510          1520          1530
  *          *          *          *          *
AAT TCG GGT AAA GCG AAA ACT CAG CAT ATC TTA TTC AGA ACG CCA TTA
TTA AGC CCA TTT CGC TTT TGA GTC GTA TAG AAT AAG TCT TGC GGT AAT
Asn Ser Gly Lys Ala Lys Thr Gln His Ile Leu Phe Arg Thr Pro Leu>
__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1540          1550          1560          1570          1580
  *          *          *          *          *
TTG ACG CCG GGA ACA GAG CAT CGT GAA CGC GTA CAA ACA GGT AAA TAT
AAC TGC GGC CCT TGT CTC GTA GCA CTT GCG CAT GTT TGT CCA TTT ATA
Leu Thr Pro Gly Thr Glu His Arg Glu Arg Val Gln Thr Gly Lys Tyr>
__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1590          1600          1610          1620          1630
  *          *          *          *          *
GAA TAT ATT ACC AAG CTC AAT ATT AAC CGT GTA GAT AGC TGG AAA ATT
CTT ATA TAA TGG TTC GAG TTA TAA TTG GCA CAT CTA TCG ACC TTT TAA
Glu Tyr Ile Thr Lys Leu Asn Ile Asn Arg Val Asp Ser Trp Lys Ile>
__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1640          1650          1660          1670          1680
  *          *          *          *          *
ACA GAT GGT GCA GCA AGT TCT ACC TTT GAT TTA ACT AAC GTT GTT CAG
TGT CTA CCA CGT CGT TCA AGA TGG AAA CTA AAT TGA TTG CAA CAA GTC
Thr Asp Gly Ala Ala Ser Ser Thr Phe Asp Leu Thr Asn Val Val Gln>
__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1690          1700          1710          1720
  *          *          *          *          *
CGT ATT GGT ATT GAA TTA GAC AAT GCT GGA AAT GTA ACT AAA ACC AAA
GCA TAA CCA TAA CTT AAT CTG TTA CGA CCT TTA CAT TGA TTT TGG TTT
Arg Ile Gly Ile Glu Leu Asp Asn Ala Gly Asn Val Thr Lys Thr Lys>
__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1730          1740          1750          1760          1770
  *          *          *          *          *
GAA ACA AAA ATT ATT GCC AAA CTT GGT GAA GGT GAT GAC AAC GTA TTT
CTT TGT TTT TAA TAA CGG TTT GAA CCA CTT CCA CTA CTG TTG CAT AAA
Glu Thr Lys Ile Ile Ala Lys Leu Gly Glu Gly Asp Asp Asn Val Phe>
__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

```

Figure 6-6

1780 1790 1800 1810 1820
 * * * * * *
 GTT GGT TCT GGT ACG ACG GAA ATT GAT GGC GGT GAA GGT TAC GAC CGA
 CAA CCA AGA CCA TGC TGC CTT TAA CTA CCG CCA CTT CCA ATG CTG GCT
 Val Gly Ser Gly Thr Thr Glu Ile Asp Gly Gly Glu Gly Tyr Asp Arg>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1830 1840 1850 1860 1870
 * * * * * *
 GTT CAC TAT AGC CGT GGA AAC TAT GGT GCT TTA ACT ATT GAT GCA ACC
 CAA GTG ATA TCG GCA CCT TTG ATA CCA CGA AAT TGA TAA CTA CGT TGG
 Val His Tyr Ser Arg Gly Asn Tyr Gly Ala Leu Thr Ile Asp Ala Thr>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1880 1890 1900 1910 1920
 * * * * * *
 AAA GAG ACC GAG CAA GGT AGT TAT ACC GTA AAT CGT TTC GTA GAA ACC
 TTT CTC TGG CTC GTT CCA TCA ATA TGG CAT TTA GCA AAG CAT CTT TGG
 Lys Glu Thr Glu Gln Gly Ser Tyr Thr Val Asn Arg Phe Val Glu Thr>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1930 1940 1950 1960
 * * * * * *
 GGT AAA GCA CTA CAC GAA GTG ACT TCA ACC CAT ACC GCA TTA GTG GGC
 CCA TTT CGT GAT GTG CTT CAC TGA AGT TGG GTA TGG CGT AAT CAC CCG
 Gly Lys Ala Leu His Glu Val Thr Ser Thr His Thr Ala Leu Val Gly>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1970 1980 1990 2000 2010
 * * * * * *
 AAC CGT GAA GAA AAA ATA GAA TAT CGT CAT AGC AAT AAC CAG CAC CAT
 TTG GCA CTT CTT TTT TAT CTT ATA GCA GTA TCG TTA TTG GTC GTG GTA
 Asn Arg Glu Glu Lys Ile Glu Tyr Arg His Ser Asn Asn Gln His His>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2020 2030 2040 2050 2060
 * * * * * *
 GCC GGT TAT TAC ACC AAA GAT ACC TTG AAA GCT GTT GAA GAA ATT ATC
 CGG CCA ATA ATG TGG TTT CTA TGG AAC TTT CGA CAA CTT CTT TAA TAG
 Ala Gly Tyr Tyr Thr Lys Asp Thr Leu Lys Ala Val Glu Glu Ile Ile>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2070 2080 2090 2100 2110
 * * * * * *
 GGT ACA TCA CAT AAC GAT ATC TTT AAA GGT AGT AAG TTC AAT GAT GCC
 CCA TGT AGT GTA TTG CTA TAG AAA TTT CCA TCA TTC AAG TTA CTA CGG
 Gly Thr Ser His Asn Asp Ile Phe Lys Gly Ser Lys Phe Asn Asp Ala>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

Figure 6-7

2120 2130 2140 2150 2160
 * * * * * * *
 TTT AAC GGT GGT GAT GGT GTC GAT ACT ATT GAC GGT AAC GAC GGC AAT
 AAA TTG CCA CCA CTA CCA CAG CTA TGA TAA CTG CCA TTG CTG CCG TTA
 Phe Asn Gly Gly Asp Gly Val Asp Thr Ile Asp Gly Asn Asp Gly Asn>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

2170 2180 2190 2200
 * * * * * * *
 GAC CGC TTA TTT GGT GGT AAA GGC GAT GAT ATT CTC GAT GGT GGA AAT
 CTG GCG AAT AAA CCA CCA TTT CCG CTA CTA TAA GAG CTA CCA CCT TTA
 Asp Arg Leu Phe Gly Gly Lys Gly Asp Asp Ile Leu Asp Gly Gly Asn>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

2210 2220 2230 2240 2250
 * * * * * * *
 GGT GAT GAT TTT ATC GAT GGC GGT AAA GGC AAC GAC CTA TTA CAC GGT
 CCA CTA CTA AAA TAG CTA CCG CCA TTT CCG TTG CTG GAT AAT GTG CCA
 Gly Asp Asp Phe Ile Asp Gly Gly Lys Gly Asn Asp Leu Leu His Gly>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

2260 2270 2280 2290 2300
 * * * * * * *
 GGC AAG GGC GAT GAT ATT TTC GTT CAC CGT AAA GGC GAT GGT AAT GAT
 CCG TTC CCG CTA CTA TAA AAG CAA GTG GCA TTT CCG CTA CCA TTA CTA
 Gly Lys Gly Asp Asp Ile Phe Val His Arg Lys Gly Asp Gly Asn Asp>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

2310 2320 2330 2340 2350
 * * * * * * *
 ATT ATT ACC GAT TCT GAC GGC AAT GAT AAA TTA TCA TTC TCT GAT TCG
 TAA TAA TGG CTA AGA CTG CCG TTA CTA TTT AAT AGT AAG AGA CTA AGC
 Ile Ile Thr Asp Ser Asp Gly Asn Asp Lys Leu Ser Phe Ser Asp Ser>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

2360 2370 2380 2390 2400
 * * * * * * *
 AAC TTA AAA GAT TTA ACA TTT GAA AAA GTT AAA CAT AAT CTT GTC ATC
 TTG AAT TTT CTA AAT TGT AAA CTT TTT CAA TTT GTA TTA GAA CAG TAG
 Asn Leu Lys Asp Leu Thr Phe Glu Lys Val Lys His Asn Leu Val Ile>
 ____a____a____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a____a____a____>

Figure 6-8

2410 2420 2430 2440
 * * * * * * *
 ACG AAT AGC AAA AAA GAG AAA GTG ACC ATT CAA AAC TGG TTC CGA GAG
 TGC TTA TCG TTT TTT CTC TTT CAC TGG TAA GTT TTG ACC AAG GCT CTC
 Thr Asn Ser Lys Lys Glu Lys Val Thr Ile Gln Asn Trp Phe Arg Glu>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2450 2460 2470 2480 2490
 * * * * * * *
 GCT GAT TTT GCT AAA GAA GTG CCT AAT TAT AAA GCA ACT AAA GAT GAG
 CGA CTA AAA CGA TTT CTT CAC GGA TTA ATA TTT CGT TGA TTT CTA CTC
 Ala Asp Phe Ala Lys Glu Val Pro Asn Tyr Lys Ala Thr Lys Asp Glu>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2500 2510 2520 2530 2540
 * * * * * * *
 AAA ATC GAA GAA ATC ATC GGT CAA AAT GGC GAG CGG ATC ACC TCA AAG
 TTT TAG CTT CTT TAG TAG CCA GTT TTA CCG CTC GCC TAG TGG AGT TTC
 Lys Ile Glu Glu Ile Ile Gly Gln Asn Gly Glu Arg Ile Thr Ser Lys>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2550 2560 2570 2580 2590
 * * * * * * *
 CAA GTT GAT GAT CTT ATC GCA AAA GGT AAC GGC AAA ATT ACC CAA GAT
 GTT CAA CTA CTA GAA TAG CGT TTT CCA TTG CCG TTT TAA TGG GTT CTA
 Gln Val Asp Asp Leu Ile Ala Lys Gly Asn Gly Lys Ile Thr Gln Asp>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2600 2610 2620 2630 2640
 * * * * * * *
 GAG CTA TCA AAA GTT GTT GAT AAC TAT GAA TTG CTC AAA CAT AGC AAA
 CTC GAT AGT TTT CAA CAA CTA TTG ATA CTT AAC GAG TTT GTA TCG TTT
 Glu Leu Ser Lys Val Val Asp Asn Tyr Glu Leu Leu Lys His Ser Lys>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2650 2660 2670 2680
 * * * * * * *
 AAT GTG ACA AAC AGC TTA GAT AAG TTA ATC TCA TCT GTA AGT GCA TTT
 TTA CAC TGT TTG TCG AAT CTA TTC AAT TAG AGT AGA CAT TCA CGT AAA
 Asn Val Thr Asn Ser Leu Asp Lys Leu Ile Ser Ser Val Ser Ala Phe>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2690 2700 2710 2720 2730
 * * * * * * *
 ACC TCG TCT AAT GAT TCG AGA AAT GTA TTA GTG GCT CCA ACT TCA ATG
 TGG AGC AGA TTA CTA AGC TCT TTA CAT AAT CAC CGA GGT TGA AGT TAC
 Thr Ser Ser Asn Asp Ser Arg Asn Val Leu Val Ala Pro Thr Ser Met>
 ___a___a___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

Figure 6-9

```

2740      2750      2760      2770      2780
  *      *      *      *      *      *      *      *
TTG GAT CAA AGT TTA TCT TCT CTT CAA TTT GCT AGG GGA TCC AGC TCT
AAC CTA GTT TCA AAT AGA AGA GAA GTT AAA CGA TCC CCT AGG TCG AGA
Leu Asp Gln Ser Leu Ser Ser Leu Gln Phe Ala Arg Gly Ser>
__a__ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]__a__a__>
                                           Ser Ser>
                                           __b__>

```

```

2790      2800      2810      2820      2830
  *      *      *      *      *      *      *      *
TCT GCC GGC TGC AAA AAC TTC TTC TGG AAA ACC TTC ACC AGC TGC TAG
AGA CGG CCG ACG TTT TTG AAG AAG ACC TTT TGG AAG TGG TCG ACG ATC
Ser>
__>
Ala Gly Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys End>
__c__c__c__c__c__c__SRIF PEPTIDE__c__c__c__c__c__c__>

```

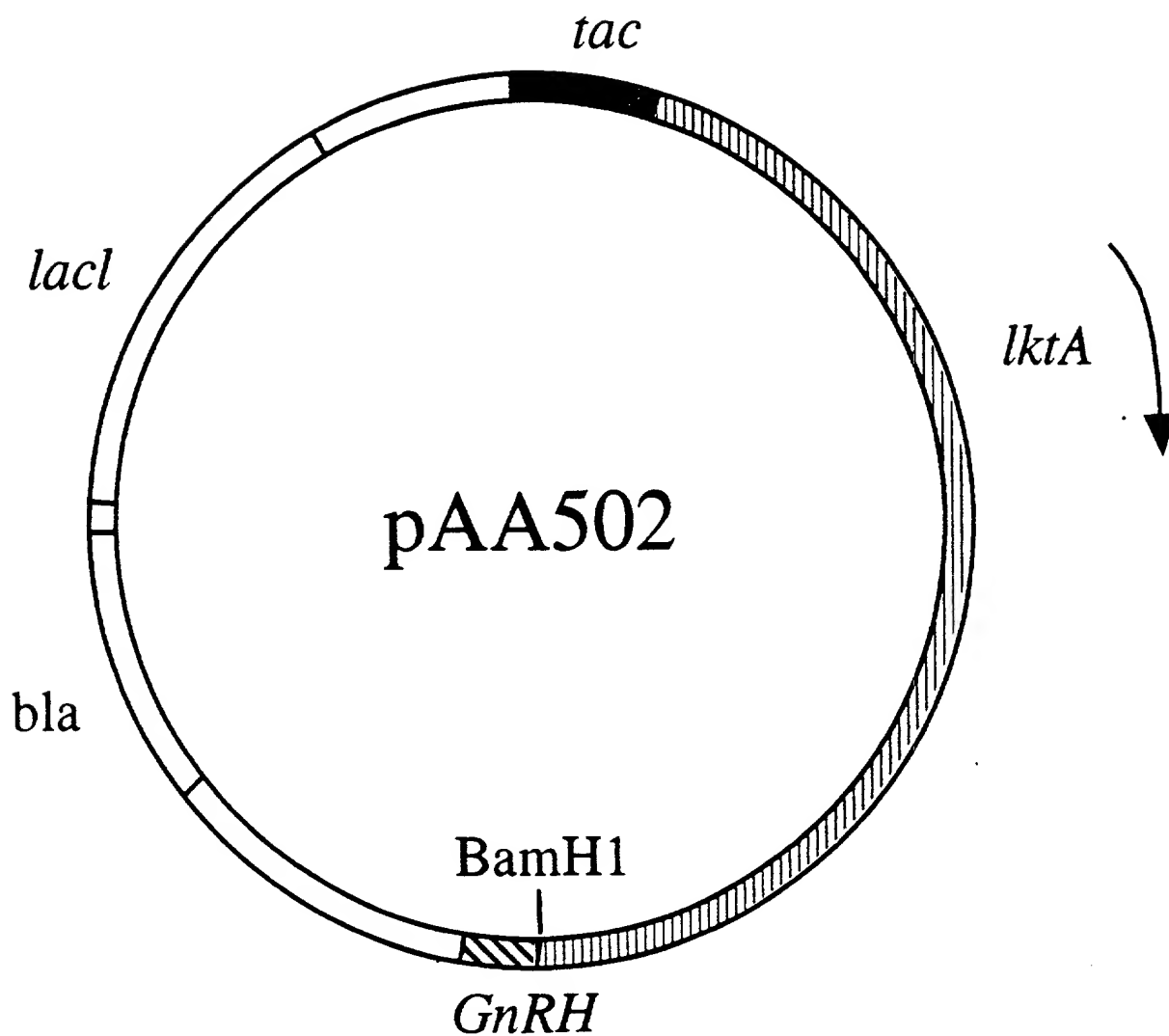
```

*
GGATCC
CCTAGG

```

Figure 6-10

93392680



tac = hybrid *trp::lac* promoter from *E. coli*
bla = beta lactamase gene (ampicillin resistance)
lktA = *Pasteurella haemolytica* structural gene
GnRH = *GnRH* gene
lacI = *E. coli* *lac* operon repressor

Figure 7

```

      10          20          30          40
    *   *       *       *       *       *       *
ATG GCT ACT GTT ATA GAT CTA AGC TTC CCA AAA ACT GGG GCA AAA AAA
TAC CGA TGA CAA TAT CTA GAT TCG AAG GGT TTT TGA CCC CGT TTT TTT
Met Ala Thr Val Ile Asp Leu Ser Phe Pro Lys Thr Gly Ala Lys Lys>
__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>


    50          60          70          80          90
    *   *       *       *       *       *       *
ATT ATC CTC TAT ATT CCC CAA AAT TAC CAA TAT GAT ACT GAA CAA GGT
TAA TAG GAG ATA TAA GGG GTT TTA ATG GTT ATA CTA TGA CTT GTT CCA
Ile Ile Leu Tyr Ile Pro Gln Asn Tyr Gln Tyr Asp Thr Glu Gln Gly>
__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>


    100         110         120         130         140
    *   *       *       *       *       *       *
AAT GGT TTA CAG GAT TTA GTC AAA GCG GCC GAA GAG TTG GGG ATT GAG
TTA CCA AAT GTC CTA AAT CAG TTT CGC CGG CTT CTC AAC CCC TAA CTC
Asn Gly Leu Gln Asp Leu Val Lys Ala Ala Glu Glu Leu Gly Ile Glu>
__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>


    150         160         170         180         190
    *   *       *       *       *       *       *
GTA CAA AGA GAA GAA CGC AAT AAT ATT GCA ACA GCT CAA ACC AGT TTA
CAT GTT TCT CTT CTT GCG TTA TTA TAA CGT TGT CGA GTT TGG TCA AAT
Val Gln Arg Glu Glu Arg Asn Asn Ile Ala Thr Ala Gln Thr Ser Leu>
__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>


    200         210         220         230         240
    *   *       *       *       *       *       *
GGC ACG ATT CAA ACC GCT ATT GGC TTA ACT GAG CGT GGC ATT GTG TTA
CCG TGC TAA GTT TGG CGA TAA CCG AAT TGA CTC GCA CCG TAA CAC AAT
Gly Thr Ile Gln Thr Ala Ile Gly Leu Thr Glu Arg Gly Ile Val Leu>
  b  b  RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

Figure 8-1

250 260 270 280
 * * * * * * *
 TCC GCT CCA CAA ATT GAT AAA TTG CTA CAG AAA ACT AAA GCA GGC CAA
 AGG CGA GGT GTT TAA CTA TTT AAC GAT GTC TTT TGA TTT CGT CCG GTT
 Ser Ala Pro Gln Ile Asp Lys Leu Leu Gln Lys Thr Lys Ala Gly Gln>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

290 300 310 320 330
 * * * * * * *
 GCA TTA GGT TCT GCC GAA AGC ATT GTA CAA AAT GCA AAT AAA GCC AAA
 CGT AAT CCA AGA CGG CTT TCG TAA CAT GTT TTA CGT TTA TTT CGG TTT
 Ala Leu Gly Ser Ala Glu Ser Ile Val Gln Asn Ala Asn Lys Ala Lys>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

340 350 360 370 380
 * * * * * * *
 ACT GTA TTA TCT GGC ATT CAA TCT ATT TTA GGC TCA GTA TTG GCT GGA
 TGA CAT AAT AGA CCG TAA GTT AGA TAA AAT CCG AGT CAT AAC CGA CCT
 Thr Val Leu Ser Gly Ile Gln Ser Ile Leu Gly Ser Val Leu Ala Gly>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

390 400 410 420 430
 * * * * * * *
 ATG GAT TTA GAT GAG GCC TTA CAG AAT AAC AGC AAC CAA CAT GCT CTT
 TAC CTA AAT CTA CTC CGG AAT GTC TTA TTG TCG TTG GTT GTA CGA GAA
 Met Asp Leu Asp Glu Ala Leu Gln Asn Asn Ser Asn Gln His Ala Leu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

440 450 460 470 480
 * * * * * * *
 GCT AAA GCT GGC TTG GAG CTA ACA AAT TCA TTA ATT GAA AAT ATT GCT
 CGA TTT CGA CCG AAC CTC GAT TGT TTA AGT AAT TAA CTT TTA TAA CGA
 Ala Lys Ala Gly Leu Glu Leu Thr Asn Ser Leu Ile Glu Asn Ile Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

490 500 510 520
 * * * * * * *
 AAT TCA GTA AAA ACA CTT GAC GAA TTN -GT GAG CAA ATT AGT CAA TTT
 TTA AGT CAT TTT TGT GAA CTG CTT AAN -CA CTC GTT TAA TCA GTT AAA
 Asn Ser Val Lys Thr Leu Asp Glu Xxx Cys Glu Gln Ile Ser Gln Phe>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

530 540 550 560 570
 * * * * * * *
 GGT TCA AAA CTA CAA AAT ATC AAA GGC TTA GGG ACT TTA GGA GAC AAA
 CCA AGT TTT GAT GTT TTA TAG TTT CCG AAT CCC TGA AAT CCT CTG TTT
 Gly Ser Lys Leu Gln Asn Ile Lys Gly Leu Gly Thr Leu Gly Asp Lys>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 8-2

580 590 600 610 620
 * * * * * * *
 CTC AAA AAT ATC GGT GGA CTT GAT AAA GCT GGC CTT GGT TTA GAT GTT
 GAG TTT TTA TAG CCA CCT GAA CTA TTT CGA CCG GAA CCA AAT CTA CAA
 Leu Lys Asn Ile Gly Gly Leu Asp Lys Ala Gly Leu Gly Leu Asp Val>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

630 640 650 660 670
 * * * * * * *
 ATC TCA GGG CTA TTA TCG GGC GCA ACA GCT GCA CTT GTA CTT GCA GAT
 TAG AGT CCC GAT AAT AGC CCG CGT TGT CGA CGT GAA CAT GAA CGT CTA
 Ile Ser Gly Leu Leu Ser Gly Ala Thr Ala Ala Leu Val Leu Ala Asp>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

680 690 700 710 720
 * * * * * * *
 AAA AAT GCT TCA ACA GCT AAA AAA GTG GGT GCG GGT TTT GAA TTG GCA
 TTT TTA CGA AGT TGT CGA TTT TTT CAC CCA CGC CCA AAA CTT AAC CGT
 Lys Asn Ala Ser Thr Ala Lys Lys Val Gly Ala Gly Phe Glu Leu Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

730 740 750 760
 * * * * * * *
 AAC CAA GTT GTT GGT AAT ATT ACC AAA GCC GTT TCT TCT TAC ATT TTA
 TTG GTT CAA CAA CCA TTA TAA TGG TTT CGG CAA AGA AGA ATG TAA AAT
 Asn Gln Val Val Gly Asn Ile Thr Lys Ala Val Ser Ser Tyr Ile Leu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

770 780 790 800 810
 * * * * * * *
 GCC CAA CGT GTT GCA GCA GGT TTA TCT TCA ACT GGG CCT GTG GCT GCT
 CGG GTT GCA CAA CGT CGT CCA AAT AGA AGT TGA CCC GGA CAC CGA CGA
 Ala Gln Arg Val Ala Ala Gly Leu Ser Ser Thr Gly Pro Val Ala Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

820 830 840 850 860
 * * * * * * *
 TTA ATT GCT TCT ACT GTT TCT CTT GCG ATT AGC CCA TTA GCA TTT GCC
 AAT TAA CGA AGA TGA CAA AGA GAA CGC TAA TCG GGT AAT CGT AAA CGG
 Leu Ile Ala Ser Thr Val Ser Leu Ala Ile Ser Pro Leu Ala Phe Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 8-3

870 880 890 900 910
 * * * * * * *
 GGT ATT GCC GAT AAA TTT AAT CAT GCA AAA AGT TTA GAG AGT TAT GCC
 CCA TAA CGG CTA TTT AAA TTA GTA CGT TTT TCA AAT CTC TCA ATA CGG
 Gly Ile Ala Asp Lys Phe Asn His Ala Lys Ser Leu Glu Ser Tyr Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

920 930 940 950 960
 * * * * * * *
 GAA CGC TTT AAA AAA TTA GGC TAT GAC GGA GAT AAT TTA TTA GCA GAA
 CTT GCG AAA TTT TTT AAT CCG ATA CTG CCT CTA TTA AAT AAT CGT CTT
 Glu Arg Phe Lys Lys Leu Gly Tyr Asp Gly Asp Asn Leu Leu Ala Glu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

970 980 990 1000
 * * * * * * *
 TAT CAG CGG GGA ACA GGG ACT ATT GAT GCA TCG GTT ACT GCA ATT AAT
 ATA GTC GCC CCT TGT CCC TGA TAA CTA CGT AGC CAA TGA CGT TAA TTA
 Tyr Gln Arg Gly Thr Gly Thr Ile Asp Ala Ser Val Thr Ala Ile Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1010 1020 1030 1040 1050
 * * * * * * *
 ACC GCA TTG GCC GCT ATT GCT GGT GGT GTG TCT GCT GCT GCA GCC GGC
 TGG CGT AAC CGG CGA TAA CGA CCA CCA CAC AGA CGA CGA CGT CGG CCG
 Thr Ala Leu Ala Ala Ile Ala Gly Gly Val Ser Ala Ala Ala Ala Gly>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1060 1070 1080 1090 1100
 * * * * * * *
 TCG GTT ATT GCT TCA CCG ATT GCC TTA TTA GTA TCT GGG ATT ACC GGT
 AGC CAA TAA CGA AGT GGC TAA CGG AAT AAT CAT AGA CCC TAA TGG CCA
 Ser Val Ile Ala Ser Pro Ile Ala Leu Leu Val Ser Gly Ile Thr Gly>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1110 1120 1130 1140 1150
 * * * * * * *
 GTA ATT TCT ACG ATT CTG CAA TAT TCT AAA CAA GCA ATG TTT GAG CAC
 CAT TAA AGA TGC TAA GAC GTT ATA AGA TTT GTT CGT TAC AAA CTC GTG
 Val Ile Ser Thr Ile Leu Gln Tyr Ser Lys Gln Ala Met Phe Glu His>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1160 1170 1180 1190 1200
 * * * * * * *
 GTT GCA AAT AAA ATT CAT AAC AAA ATT GTA GAA TGG GAA AAA AAT AAT
 CAA CGT TTA TTT TAA GTA TTG TTT TAA CAT CTT ACC CTT TTT TTA TTA
 Val Ala Asn Lys Ile His Asn Lys Ile Val Glu Trp Glu Lys Asn Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 8-4

1210 1220 1230 1240
 * * * * * * *
 CAC GGT AAG AAC TAC TTT GAA AAT GGT TAC GAT GCC CGT TAT CTT GCG
 GTG CCA TTC TTG ATG AAA CTT TTA CCA ATG CTA CGG GCA ATA GAA CGC
 His Gly Lys Asn Tyr Phe Glu Asn Gly Tyr Asp Ala Arg Tyr Leu Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1250 1260 1270 1280 1290
 * * * * * * *
 AAT TTA CAA GAT AAT ATG AAA TTC TTA CTG AAC TTA AAC AAA GAG TTA
 TTA AAT GTT CTA TTA TAC TTT AAG AAT GAC TTG AAT TTG TTT CTC AAT
 Asn Leu Gln Asp Asn Met Lys Phe Leu Leu Asn Leu Asn Lys Glu Leu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1300 1310 1320 1330 1340
 * * * * * * *
 CAG GCA GAA CGT GTC ATC GCT ATT ACT CAG CAG CAA TGG GAT AAC AAC
 GTC CGT CTT GCA CAG TAG CGA TAA TGA GTC GTC GTT ACC CTA TTG TTG
 Gln Ala Glu Arg Val Ile Ala Ile Thr Gln Gln Gln Trp Asp Asn Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1350 1360 1370 1380 1390
 * * * * * * *
 ATT GGT GAT TTA GCT GGT ATT AGC CGT TTA GGT GAA AAA GTC CTT AGT
 TAA CCA CTA AAT CGA CCA TAA TCG GCA AAT CCA CTT TTT CAG GAA TCA
 Ile Gly Asp Leu Ala Gly Ile Ser Arg Leu Gly Glu Lys Val Leu Ser>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1400 1410 1420 1430 1440
 * * * * * * *
 GGT AAA GCC TAT GTG GAT GCG TTT GAA GAA GGC AAA CAC ATT AAA GCC
 CCA TTT CGG ATA CAC CTA CGC AAA CTT CTT CCG TTT GTG TAA TTT CGG
 Gly Lys Ala Tyr Val Asp Ala Phe Glu Glu Gly Lys His Ile Lys Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1450 1460 1470 1480
 * * * * * * *
 GAT AAA TTA GTA CAG TTG GAT TCG GCA AAC GGT ATT ATT GAT GTG AGT
 CTA TTT AAT CAT GTC AAC CTA AGC CGT TTG CCA TAA TAA CTA CAC TCA
 Asp Lys Leu Val Gln Leu Asp Ser Ala Asn Gly Ile Ile Asp Val Ser>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 8-5

1490 1500 1510 1520 1530
 * * * * * * * *
 AAT TCG GGT AAA GCG AAA ACT CAG CAT ATC TTA TTC AGA ACG CCA TTA
 TTA AGC CCA TTT CGC TTT TGA GTC GTA TAG AAT AAG TCT TGC GGT AAT
 Asn Ser Gly Lys Ala Lys Thr Gln His Ile Leu Phe Arg Thr Pro Leu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1540 1550 1560 1570 1580
 * * * * * * *
 TTG ACG CCG GGA ACA GAG CAT CGT GAA CGC GTA CAA ACA GGT AAA TAT
 AAC TGC GGC CCT TGT CTC GTA GCA CTT GCG CAT GTT TGT CCA TTT ATA
 Leu Thr Pro Gly Thr Glu His Arg Glu Arg Val Gln Thr Gly Lys Tyr>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1590 1600 1610 1620 1630
 * * * * * * *
 GAA TAT ATT ACC AAG CTC AAT ATT AAC CGT GTA GAT AGC TGG AAA ATT
 CTT ATA TAA TGG TTC GAG TTA TAA TTG GCA CAT CTA TCG ACC TTT TAA
 Glu Tyr Ile Thr Lys Leu Asn Ile Asn Arg Val Asp Ser Trp Lys Ile>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1640 1650 1660 1670 1680
 * * * * * * *
 ACA GAT GGT GCA GCA AGT TCT ACC TTT GAT TTA ACT AAC GTT GTT CAG
 TGT CTA CCA CGT CGT TCA AGA TGG AAA CTA AAT TGA TTG CAA CAA GTC
 Thr Asp Gly Ala Ala Ser Ser Thr Phe Asp Leu Thr Asn Val Val Gln>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1690 1700 1710 1720
 * * * * * * *
 CGT ATT GGT ATT GAA TTA GAC AAT GCT GGA AAT GTA ACT AAA ACC AAA
 GCA TAA CCA TAA CTT AAT CTG TTA CGA CCT TTA CAT TGA TTT TGG TTT
 Arg Ile Gly Ile Glu Leu Asp Asn Ala Gly Asn Val Thr Lys Thr Lys>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1730 1740 1750 1760 1770
 * * * * * * *
 GAA ACA AAA ATT ATT GCC AAA CTT GGT GAA GGT GAT GAC AAC GTA TTT
 CTT TGT TTT TAA TAA CGG TTT GAA CCA CTT CCA CTA CTG TTG CAT AAA
 Glu Thr Lys Ile Ile Ala Lys Leu Gly Glu Gly Asp Asp Asn Val Phe>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1780 1790 1800 1810 1820
 * * * * * * *
 GTT GGT TCT GGT ACG ACG GAA ATT GAT GGC GGT GAA GGT TAC GAC CGA
 CAA CCA AGA CCA TGC TGC CTT TAA CTA CCG CCA CTT CCA ATG CTG GCT
 Val Gly Ser Gly Thr Thr Glu Ile Asp Gly Gly Glu Gly Tyr Asp Arg>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 8-6

08976566-44497

2120 2130 2140 2150 2160
 * * * * * * *
 TTT AAC GGT GGT GAT GGT GTC GAT ACT ATT GAC GGT AAC GAC GGC AAT
 AAA TTG CCA CCA CTA CCA CAG CTA TGA TAA CTG CCA TTG CTG CCG TTA
 Phe Asn Gly Gly Asp Gly Val Asp Thr Ile Asp Gly Asn Asp Gly Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2170 2180 2190 2200
 * * * * * * *
 GAC CGC TTA TTT GGT GGT AAA GGC GAT GAT ATT CTC GAT GGT GGA AAT
 CTG GCG AAT AAA CCA CCA TTT CCG CTA CTA TAA GAG CTA CCA CCT TTA
 Asp Arg Leu Phe Gly Gly Lys Gly Asp Asp Ile Leu Asp Gly Gly Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2210 2220 2230 2240 2250
 * * * * * * *
 GGT GAT GAT TTT ATC GAT GGC GGT AAA GGC AAC GAC CTA TTA CAC GGT
 CCA CTA CTA AAA TAG CTA CCG CCA TTT CCG TTG CTG GAT AAT GTG CCA
 Gly Asp Asp Phe Ile Asp Gly Gly Lys Gly Asn Asp Leu Leu His Gly>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2260 2270 2280 2290 2300
 * * * * * * *
 GGC AAG GGC GAT GAT ATT TTC GTT CAC CGT AAA GGC GAT GGT AAT GAT
 CCG TTC CCG CTA CTA TAA AAG CAA GTG GCA TTT CCG CTA CCA TTA CTA
 Gly Lys Gly Asp Asp Ile Phe Val His Arg Lys Gly Asp Gly Asn Asp>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2310 2320 2330 2340 2350
 * * * * * * *
 ATT ATT ACC GAT TCT GAC GGC AAT GAT AAA TTA TCA TTC TCT GAT TCG
 TAA TAA TGG CTA AGA CTG CCG TTA CTA TTT AAT AGT AAG AGA CTA AGC
 Ile Ile Thr Asp Ser Asp Gly Asn Asp Lys Leu Ser Phe Ser Asp Ser>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2360 2370 2380 2390 2400
 * * * * * * *
 AAC TTA AAA GAT TTA ACA TTT GAA AAA GTT AAA CAT AAT CTT GTC ATC
 TTG AAT TTT CTA AAT TGT AAA CTT TTT CAA TTT GTA TTA GAA CAG TAG
 Asn Leu Lys Asp Leu Thr Phe Glu Lys Val Lys His Asn Leu Val Ile>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2410 2420 2430 2440
 * * * * * * *
 ACG AAT AGC AAA AAA GAG AAA GTG ACC ATT CAA AAC TGG TTC CGA GAG
 TGC TTA TCG TTT TTT CTC TTT CAC TGG TAA GTT TTG ACC AAG GCT CTC
 Thr Asn Ser Lys Lys Glu Lys Val Thr Ile Gln Asn Trp Phe Arg Glu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 8-8

2450 2460 2470 2480 2490
 * * * * * * * *
 GCT GAT TTT GCT AAA GAA GTG CCT AAT TAT AAA GCA ACT AAA GAT GAG
 CGA CTA AAA CGA TTT CTT CAC GGA TTA ATA TTT CGT TGA TTT CTA CTC
 Ala Asp Phe Ala Lys Glu Val Pro Asn Tyr Lys Ala Thr Lys Asp Glu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2500 2510 2520 2530 2540
 * * * * * * *
 AAA ATC GAA GAA ATC ATC GGT CAA AAT GGC GAG CGG ATC ACC TCA AAG
 TTT TAG CTT CTT TAG TAG CCA GTT TTA CCG CTC GCC TAG TGG AGT TTC
 Lys Ile Glu Glu Ile Ile Gly Gln Asn Gly Glu Arg Ile Thr Ser Lys>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2550 2560 2570 2580 2590
 * * * * * * *
 CAA GTT GAT GAT CTT ATC GCA AAA GGT AAC GGC AAA ATT ACC CAA GAT
 GTT CAA CTA CTA GAA TAG CGT TTT CCA TTG CCG TTT TAA TGG GTT CTA
 Gln Val Asp Asp Leu Ile Ala Lys Gly Asn Gly Lys Ile Thr Gln Asp>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2600 2610 2620 2630 2640
 * * * * * * *
 GAG CTA TCA AAA GTT GTT GAT AAC TAT GAA TTG CTC AAA CAT AGC AAA
 CTC GAT AGT TTT CAA CAA CTA TTG ATA CTT AAC GAG TTT GTA TCG TTT
 Glu Leu Ser Lys Val Val Asp Asn Tyr Glu Leu Leu Lys His Ser Lys>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2650 2660 2670 2680
 * * * * * * *
 AAT GTG ACA AAC AGC TTA GAT AAG TTA ATC TCA TCT GTA AGT GCA TTT
 TTA CAC TGT TTG TCG AAT CTA TTC AAT TAG AGT AGA CAT TCA CGT AAA
 Asn Val Thr Asn Ser Leu Asp Lys Leu Ile Ser Ser Val Ser Ala Phe>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2690 2700 2710 2720 2730
 * * * * * * *
 ACC TCG TCT AAT GAT TCG AGA AAT GTA TTA GTG GCT CCA ACT TCA ATG
 TGG AGC AGA TTA CTA AGC TCT TTA CAT AAT CAC CGA GGT TGA AGT TAC
 Thr Ser Ser Asn Asp Ser Arg Asn Val Leu Val Ala Pro Thr Ser Met>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 8-9

```

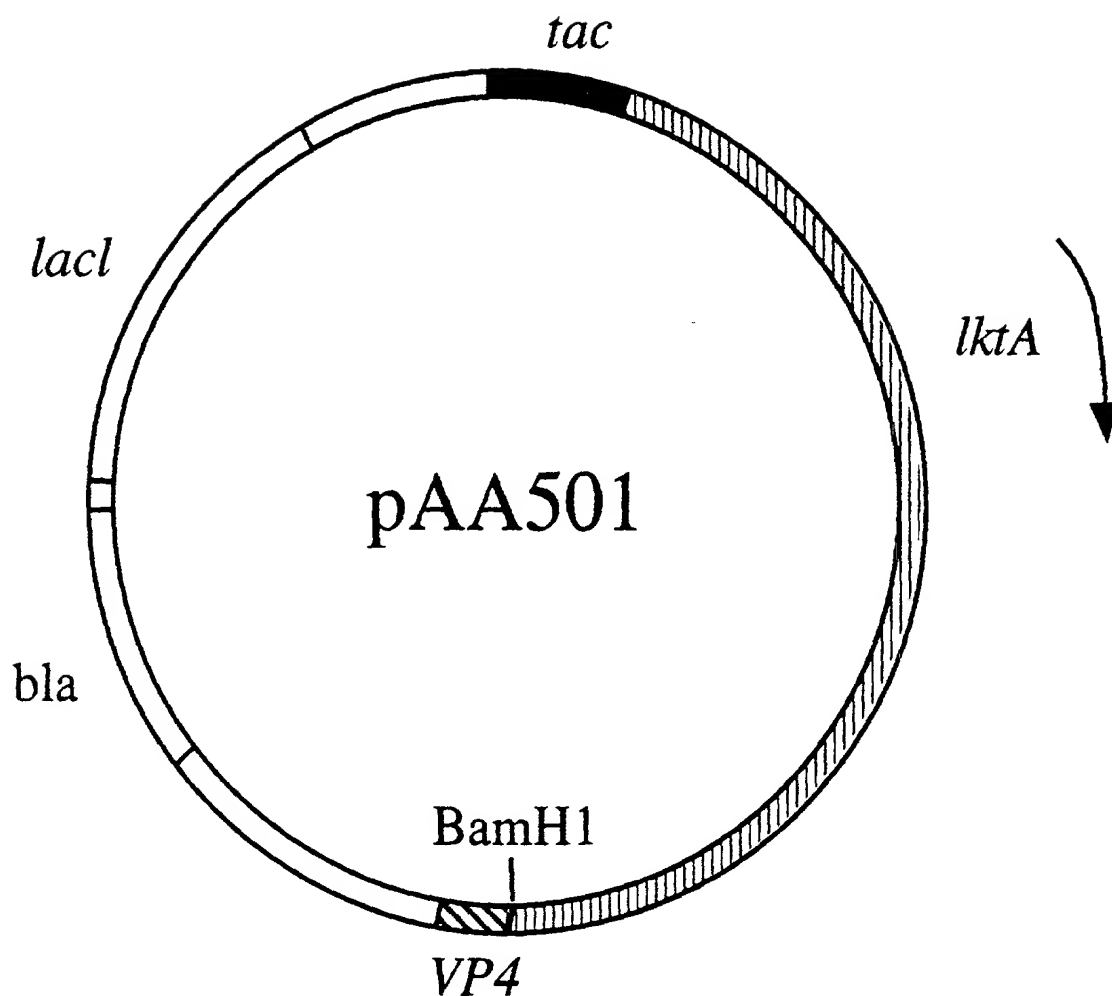
2740          2750          2760          2770          2780
  *      *      *      *      *      *      *      *      *
TTG GAT CAA AGT TTA TCT TCT CTT CAA TTT GCT AGG GGA TCT CAG CAT
AAC CTA GTT TCA AAT AGA AGA GAA GTT AAA CGA TCC CCT AGA GTC GTA
                                     Gln His>
Leu Asp Gln Ser Leu Ser Ser Leu Gln Phe Ala Arg Gly Ser>___a___>
___b___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___>

2790          2800          2810
  *      *      *      *      *      *      *
TGG AGC TAC GGC CTG CGC CCT GGC TAA GGATCC
ACC TCG ATG CCG GAC GCG GGA CCG ATT CCTAGG
Trp Ser Tyr Gly Leu Arg Pro Gly End>
___a___a___a___GNRH___a___a___a___>

```

Figure 8-10

0897636-1449



tac = hybrid *trp::lac* promoter from *E. coli*
bla = beta lactamase gene (ampicillin resistance)
lktA = *Pasteurella haemolytica* structural gene
VP4 = Bovine rotavirus VP4(232 - 255) gene
lacI = *E. coli* lac operon repressor

Figure 9

```

      10      20      30      40
      *      *      *      *      *      *
ATG GCT ACT GTT ATA GAT CTA AGC TTC CCA AAA ACT GGG GCA AAA AAA
TAC CGA TGA CAA TAT CTA GAT TCG AAG GGT TTT TGA CCC CGT TTT TTT
Met Ala Thr Val Ile Asp Leu Ser Phe Pro Lys Thr Gly Ala Lys Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

50      60      70      80      90
      *      *      *      *      *      *
ATT ATC CTC TAT ATT CCC CAA AAT TAC CAA TAT GAT ACT GAA CAA GGT
TAA TAG GAG ATA TAA GGG GTT TTA ATG GTT ATA CTA TGA CTT GTT CCA
Ile Ile Leu Tyr Ile Pro Gln Asn Tyr Gln Tyr Asp Thr Glu Gln Gly>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

100      110      120      130      140
      *      *      *      *      *      *
AAT GGT TTA CAG GAT TTA GTC AAA GCG GCC GAA GAG TTG GGG ATT GAG
TTA CCA AAT GTC CTA AAT CAG TTT CGC CGG CTT CTC AAC CCC TAA CTC
Asn Gly Leu Gln Asp Leu Val Lys Ala Ala Glu Glu Leu Gly Ile Glu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

150      160      170      180      190
      *      *      *      *      *      *
GTA CAA AGA GAA GAA CGC AAT AAT ATT GCA ACA GCT CAA ACC AGT TTA
CAT GTT TCT CTT CTT GCG TTA TTA TAA CGT TGT CGA GTT TGG TCA AAT
Val Gln Arg Glu Glu Arg Asn Asn Ile Ala Thr Ala Gln Thr Ser Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

200      210      220      230      240
      *      *      *      *      *      *
GGC ACG ATT CAA ACC GCT ATT GGC TTA ACT GAG CGT GGC ATT GTG TTA
CCG TGC TAA GTT TGG CGA TAA CCG AAT TGA CTC GCA CCG TAA CAC AAT
Gly Thr Ile Gln Thr Ala Ile Gly Leu Thr Glu Arg Gly Ile Val Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

Figure 10-1

```

      250      260      270      280
      *      *      *      *      *      *      *      *
TCC GCT CCA CAA ATT GAT AAA TTG CTA CAG AAA ACT AAA GCA GGC CAA
AGG CGA GGT GTT TAA CTA TTT AAC GAT GTC TTT TGA TTT CGT CCG GTT
Ser Ala Pro Gln Ile Asp Lys Leu Leu Gln Lys Thr Lys Ala Gly Gln>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

290      300      310      320      330
      *      *      *      *      *      *      *      *
GCA TTA GGT TCT GCC GAA AGC ATT GTA CAA AAT GCA AAT AAA GCC AAA
CGT AAT CCA AGA CGG CTT TCG TAA CAT GTT TTA CGT TTA TTT CGG TTT
Ala Leu Gly Ser Ala Glu Ser Ile Val Gln Asn Ala Asn Lys Ala Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      340      350      360      370      380
      *      *      *      *      *      *      *      *
ACT GTA TTA TCT GGC ATT CAA TCT ATT TTA GGC TCA GTA TTG GCT GGA
TGA CAT AAT AGA CCG TAA GTT AGA TAA AAT CCG AGT CAT AAC CGA CCT
Thr Val Leu Ser Gly Ile Gln Ser Ile Leu Gly Ser Val Leu Ala Gly>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      390      400      410      420      430
      *      *      *      *      *      *      *      *
ATG GAT TTA GAT GAG GCC TTA CAG AAT AAC AGC AAC CAA CAT GCT CTT
TAC CTA AAT CTA CTC CGG AAT GTC TTA TTG TCG TTG GTT GTA CGA GAA
Met Asp Leu Asp Glu Ala Leu Gln Asn Asn Ser Asn Gln His Ala Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      440      450      460      470      480
      *      *      *      *      *      *      *      *
GCT AAA GCT GGC TTG GAG CTA ACA AAT TCA TTA ATT GAA AAT ATT GCT
CGA TTT CGA CCG AAC CTC GAT TGT TTA AGT AAT TAA CTT TTA TAA CGA
Ala Lys Ala Gly Leu Glu Leu Thr Asn Ser Leu Ile Glu Asn Ile Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      490      500      510      520
      *      *      *      *      *      *      *      *
AAT TCA GTA AAA ACA CTT GAC GAA TTN -GT GAG CAA ATT AGT CAA TTT
TTA AGT CAT TTT TGT GAA CTG CTT AAN -CA CTC GTT TAA TCA GTT AAA
Asn Ser Val Lys Thr Leu Asp Glu Xxx Cys Glu Gln Ile Ser Gln Phe>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

530      540      550      560      570
      *      *      *      *      *      *      *      *
GGT TCA AAA CTA CAA AAT ATC AAA GGC TTA GGG ACT TTA GGA GAC AAA
CCA AGT TTT GAT GTT TTA TAG TTT CCG AAT CCC TGA AAT CCT CTG TTT
Gly Ser Lys Leu Gln Asn Ile Lys Gly Leu Gly Thr Leu Gly Asp Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

Figure 10-2

580 590 600 610 620
 * * * * *
 CTC AAA AAT ATC GGT GGA CTT GAT AAA GCT GGC CTT GGT TTA GAT GTT
 GAG TTT TTA TAG CCA CCT GAA CTA TTT CGA CCG GAA CCA AAT CTA CAA
 Leu Lys Asn Ile Gly Gly Leu Asp Lys Ala Gly Leu Gly Leu Asp Val>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

630 640 650 660 670
 * * * * *
 ATC TCA GGG CTA TTA TCG GGC GCA ACA GCT GCA CTT GTA CTT GCA GAT
 TAG AGT CCC GAT AAT AGC CCG CGT TGT CGA CGT GAA CAT GAA CGT CTA
 Ile Ser Gly Leu Leu Ser Gly Ala Thr Ala Ala Leu Val Leu Ala Asp>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

680 690 700 710 720
 * * * * *
 AAA AAT GCT TCA ACA GCT AAA AAA GTG GGT GCG GGT TTT GAA TTG GCA
 TTT TTA CGA AGT TGT CGA TTT TTT CAC CCA CGC CCA AAA CTT AAC CGT
 Lys Asn Ala Ser Thr Ala Lys Lys Val Gly Ala Gly Phe Glu Leu Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

730 740 750 760
 * * * * *
 AAC CAA GTT GTT GGT AAT ATT ACC AAA GCC GTT TCT TCT TAC ATT TTA
 TTG GTT CAA CAA CCA TTA TAA TGG TTT CGG CAA AGA AGA ATG TAA AAT
 Asn Gln Val Val Gly Asn Ile Thr Lys Ala Val Ser Ser Tyr Ile Leu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

770 780 790 800 810
 * * * * *
 GCC CAA CGT GTT GCA GCA GGT TTA TCT TCA ACT GGG CCT GTG GCT GCT
 CGG GTT GCA CAA CGT CGT CCA AAT AGA AGT TGA CCC GGA CAC CGA CGA
 Ala Gln Arg Val Ala Ala Gly Leu Ser Ser Thr Gly Pro Val Ala Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

820 830 840 850 860
 * * * * *
 TTA ATT GCT TCT ACT GTT TCT CTT GCG ATT AGC CCA TTA GCA TTT GCC
 AAT TAA CGA AGA TGA CAA AGA GAA CGC TAA TCG GGT AAT CGT AAA CGG
 Leu Ile Ala Ser Thr Val Ser Leu Ala Ile Ser Pro Leu Ala Phe Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 10-3

870 880 890 900 910
 * * * * * * * * *
 GGT ATT GCC GAT AAA TTT AAT CAT GCA AAA AGT TTA GAG AGT TAT GCC
 CCA TAA CGG CTA TTT AAA TTA GTA CGT TTT TCA AAT CTC TCA ATA CGG
 Gly Ile Ala Asp Lys Phe Asn His Ala Lys Ser Leu Glu Ser Tyr Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

920 930 940 950 960
 * * * * * * * * *
 GAA CGC TTT AAA AAA TTA GGC TAT GAC GGA GAT AAT TTA TTA GCA GAA
 CTT GCG AAA TTT TTT AAT CCG ATA CTG CCT CTA TTA AAT AAT CGT CTT
 Glu Arg Phe Lys Lys Leu Gly Tyr Asp Gly Asp Asn Leu Leu Ala Glu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

970 980 990 1000
 * * * * * * * * *
 TAT CAG CGG GGA ACA GGG ACT ATT GAT GCA TCG GTT ACT GCA ATT AAT
 ATA GTC GCC CCT TGT CCC TGA TAA CTA CGT AGC CAA TGA CGT TAA TTA
 Tyr Gln Arg Gly Thr Gly Thr Ile Asp Ala Ser Val Thr Ala Ile Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1010 1020 1030 1040 1050
 * * * * * * * * *
 ACC GCA TTG GCC GCT ATT GCT GGT GGT GTG TCT GCT GCT GCA GCC GGC
 TGG CGT AAC CGG CGA TAA CGA CCA CCA CAC AGA CGA CGA CGT CGG CCG
 Thr Ala Leu Ala Ala Ile Ala Gly Gly Val Ser Ala Ala Ala Ala Gly>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1060 1070 1080 1090 1100
 * * * * * * * * *
 TCG GTT ATT GCT TCA CCG ATT GCC TTA TTA GTA TCT GGG ATT ACC GGT
 AGC CAA TAA CGA AGT GGC TAA CGG AAT AAT CAT AGA CCC TAA TGG CCA
 Ser Val Ile Ala Ser Pro Ile Ala Leu Leu Val Ser Gly Ile Thr Gly>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1110 1120 1130 1140 1150
 * * * * * * * * *
 GTA ATT TCT ACG ATT CTG CAA TAT TCT AAA CAA GCA ATG TTT GAG CAC
 CAT TAA AGA TGC TAA GAC GTT ATA AGA TTT GTT CGT TAC AAA CTC GTG
 Val Ile Ser Thr Ile Leu Gln Tyr Ser Lys Gln Ala Met Phe Glu His>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1160 1170 1180 1190 1200
 * * * * * * * * *
 GTT GCA AAT AAA ATT CAT AAC AAA ATT GTA GAA TGG GAA AAA AAT AAT
 CAA CGT TTA TTT TAA GTA TTG TTT TAA CAT CTT ACC CTT TTT TTA TTA
 Val Ala Asn Lys Ile His Asn Lys Ile Val Glu Trp Glu Lys Asn Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 10-4

1210 1220 1230 1240
 * * * * * * *
 CAC GGT AAG AAC TAC TTT GAA AAT GGT TAC GAT GCC CGT TAT CTT GCG
 GTG CCA TTC TTG ATG AAA CTT TTA CCA ATG CTA CGG GCA ATA GAA CGC
 His Gly Lys Asn Tyr Phe Glu Asn Gly Tyr Asp Ala Arg Tyr Leu Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1250 1260 1270 1280 1290
 * * * * * * *
 AAT TTA CAA GAT AAT ATG AAA TTC TTA CTG AAC TTA AAC AAA GAG TTA
 TTA AAT GTT CTA TTA TAC TTT AAG AAT GAC TTG AAT TTG TTT CTC AAT
 Asn Leu Gln Asp Asn Met Lys Phe Leu Leu Asn Leu Asn Lys Glu Leu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1300 1310 1320 1330 1340
 * * * * * * *
 CAG GCA GAA CGT GTC ATC GCT ATT ACT CAG CAG CAA TGG GAT AAC AAC
 GTC CGT CTT GCA CAG TAG CGA TAA TGA GTC GTC GTT ACC CTA TTG TTG
 Gln Ala Glu Arg Val Ile Ala Ile Thr Gln Gln Gln Trp Asp Asn Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1350 1360 1370 1380 1390
 * * * * * * *
 ATT GGT GAT TTA GCT GGT ATT AGC CGT TTA GGT GAA AAA GTC CTT AGT
 TAA CCA CTA AAT CGA CCA TAA TCG GCA AAT CCA CTT TTT CAG GAA TCA
 Ile Gly Asp Leu Ala Gly Ile Ser Arg Leu Gly Glu Lys Val Leu Ser>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1400 1410 1420 1430 1440
 * * * * * * *
 GGT AAA GCC TAT GTG GAT GCG TTT GAA GAA GGC AAA CAC ATT AAA GCC
 CCA TTT CGG ATA CAC CTA CGC AAA CTT CTT CCG TTT GTG TAA TTT CGG
 Gly Lys Ala Tyr Val Asp Ala Phe Glu Glu Gly Lys His Ile Lys Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1450 1460 1470 1480
 * * * * * * *
 GAT AAA TTA GTA CAG TTG GAT TCG GCA AAC GGT ATT ATT GAT GTG AGT
 CTA TTT AAT CAT GTC AAC CTA AGC CGT TTG CCA TAA TAA CTA CAC TCA
 Asp Lys Leu Val Gln Leu Asp Ser Ala Asn Gly Ile Ile Asp Val Ser>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 10-5

1490 1500 1510 1520 1530
 * * * * * *
 AAT TCG GGT AAA GCG AAA ACT CAG CAT ATC TTA TTC AGA ACG CCA TTA
 TTA AGC CCA TTT CGC TTT TGA GTC GTA TAG AAT AAG TCT TGC GGT AAT
 Asn Ser Gly Lys Ala Lys Thr Gln His Ile Leu Phe Arg Thr Pro Leu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1540 1550 1560 1570 1580
 * * * * * *
 TTG ACG CCG GGA ACA GAG CAT CGT GAA CGC GTA CAA ACA GGT AAA TAT
 AAC TGC GGC CCT TGT CTC GTA GCA CTT GCG CAT GTT TGT CCA TTT ATA
 Leu Thr Pro Gly Thr Glu His Arg Glu Arg Val Gln Thr Gly Lys Tyr>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1590 1600 1610 1620 1630
 * * * * * *
 GAA TAT ATT ACC AAG CTC AAT ATT AAC CGT GTA GAT AGC TGG AAA ATT
 CTT ATA TAA TGG TTC GAG TTA TAA TTG GCA CAT CTA TCG ACC TTT TAA
 Glu Tyr Ile Thr Lys Leu Asn Ile Asn Arg Val Asp Ser Trp Lys Ile>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1640 1650 1660 1670 1680
 * * * * * *
 ACA GAT GGT GCA GCA AGT TCT ACC TTT GAT TTA ACT AAC GTT GTT CAG
 TGT CTA CCA CGT CGT TCA AGA TGG AAA CTA AAT TGA TTG CAA CAA GTC
 Thr Asp Gly Ala Ala Ser Ser Thr Phe Asp Leu Thr Asn Val Val Gln>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1690 1700 1710 1720
 * * * * * *
 CGT ATT GGT ATT GAA TTA GAC AAT GCT GGA AAT GTA ACT AAA ACC AAA
 GCA TAA CCA TAA CTT AAT CTG TTA CGA CCT TTA CAT TGA TTT TGG TTT
 Arg Ile Gly Ile Glu Leu Asp Asn Ala Gly Asn Val Thr Lys Thr Lys>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1730 1740 1750 1760 1770
 * * * * * *
 GAA ACA AAA ATT ATT GCC AAA CTT GGT GAA GGT GAT GAC AAC GTA TTT
 CTT TGT TTT TAA TAA CGG TTT GAA CCA CTT CCA CTA CTG TTG CAT AAA
 Glu Thr Lys Ile Ile Ala Lys Leu Gly Glu Gly Asp Asp Asn Val Phe>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1780 1790 1800 1810 1820
 * * * * * *
 GTT GGT TCT GGT ACG ACG GAA ATT GAT GGC GGT GAA GGT TAC GAC CGA
 CAA CCA AGA CCA TGC TGC CTT TAA CTA CCG CCA CTT CCA ATG CTG GCT
 Val Gly Ser Gly Thr Thr Glu Ile Asp Gly Gly Glu Gly Tyr Asp Arg>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 10-6

1830 1840 1850 1860 1870
 * * * * *
 GTT CAC TAT AGC CGT GGA AAC TAT GGT GCT TTA ACT ATT GAT GCA ACC
 CAA GTG ATA TCG GCA CCT TTG ATA CCA CGA AAT TGA TAA CTA CGT TGG
 Val His Tyr Ser Arg Gly Asn Tyr Gly Ala Leu Thr Ile Asp Ala Thr>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1880 1890 1900 1910 1920
 * * * * *
 AAA GAG ACC GAG CAA GGT AGT TAT ACC GTA AAT CGT TTC GTA GAA ACC
 TTT CTC TGG CTC GTT CCA TCA ATA TGG CAT TTA GCA AAG CAT CTT TGG
 Lys Glu Thr Glu Gln Gly Ser Tyr Thr Val Asn Arg Phe Val Glu Thr>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1930 1940 1950 1960
 * * * * *
 GGT AAA GCA CTA CAC GAA GTG ACT TCA ACC CAT ACC GCA TTA GTG GGC
 CCA TTT CGT GAT GTG CTT CAC TGA AGT TGG GTA TGG CGT AAT CAC CCG
 Gly Lys Ala Leu His Glu Val Thr Ser Thr His Thr Ala Leu Val Gly>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1970 1980 1990 2000 2010
 * * * * *
 AAC CGT GAA GAA AAA ATA GAA TAT CGT CAT AGC AAT AAC CAG CAC CAT
 TTG GCA CTT CTT TTT TAT CTT ATA GCA GTA TCG TTA TTG GTC GTG GTA
 Asn Arg Glu Glu Lys Ile Glu Tyr Arg His Ser Asn Asn Gln His His>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2020 2030 2040 2050 2060
 * * * * *
 GCC GGT TAT TAC ACC AAA GAT ACC TTG AAA GCT GTT GAA GAA ATT ATC
 CGG CCA ATA ATG TGG TTT CTA TGG AAC TTT CGA CAA CTT CTT TAA TAG
 Ala Gly Tyr Tyr Thr Lys Asp Thr Leu Lys Ala Val Glu Glu Ile Ile>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2070 2080 2090 2100 2110
 * * * * *
 GGT ACA TCA CAT AAC GAT ATC TTT AAA GGT AGT AAG TTC AAT GAT GCC
 CCA TGT AGT GTA TTG CTA TAG AAA TTT CCA TCA TTC AAG TTA CTA CGG
 Gly Thr Ser His Asn Asp Ile Phe Lys Gly Ser Lys Phe Asn Asp Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 10-7

2120 2130 2140 2150 2160
 * * * * * *
 TTT AAC GGT GGT GAT GGT GTC GAT ACT ATT GAC GGT AAC GAC GGC AAT
 AAA TTG CCA CCA CTA CCA CAG CTA TGA TAA CTG CCA TTG CTG CCG TTA
 Phe Asn Gly Gly Asp Gly Val Asp Thr Ile Asp Gly Asn Asp Gly Asn>
 ____b____b____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b____b____b____>

2170 2180 2190 2200
 * * * * * *
 GAC CGC TTA TTT GGT GGT AAA GGC GAT GAT ATT CTC GAT GGT GGA AAT
 CTG GCG AAT AAA CCA CCA TTT CCG CTA CTA TAA GAG CTA CCA CCT TTA
 Asp Arg Leu Phe Gly Gly Lys Gly Asp Asp Ile Leu Asp Gly Gly Asn>
 ____b____b____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b____b____b____>

2210 2220 2230 2240 2250
 * * * * * *
 GGT GAT GAT TTT ATC GAT GGC GGT AAA GGC AAC GAC CTA TTA CAC GGT
 CCA CTA CTA AAA TAG CTA CCG CCA TTT CCG TTG CTG GAT AAT GTG CCA
 Gly Asp Asp Phe Ile Asp Gly Gly Lys Gly Asn Asp Leu Leu His Gly>
 ____b____b____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b____b____b____>

2260 2270 2280 2290 2300
 * * * * * *
 GGC AAG GGC GAT GAT ATT TTC GTT CAC CGT AAA GGC GAT GGT AAT GAT
 CCG TTC CCG CTA CTA TAA AAG CAA GTG GCA TTT CCG CTA CCA TTA CTA
 Gly Lys Gly Asp Asp Ile Phe Val His Arg Lys Gly Asp Gly Asn Asp>
 ____b____b____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b____b____b____>

2310 2320 2330 2340 2350
 * * * * * *
 ATT ATT ACC GAT TCT GAC GGC AAT GAT AAA TTA TCA TTC TCT GAT TCG
 TAA TAA TGG CTA AGA CTG CCG TTA CTA TTT AAT AGT AAG AGA CTA AGC
 Ile Ile Thr Asp Ser Asp Gly Asn Asp Lys Leu Ser Phe Ser Asp Ser>
 ____b____b____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b____b____b____>

2360 2370 2380 2390 2400
 * * * * * *
 AAC TTA AAA GAT TTA ACA TTT GAA AAA GTT AAA CAT AAT CTT GTC ATC
 TTG AAT TTT CTA AAT TGT AAA CTT TTT CAA TTT GTA TTA GAA CAG TAG
 Asn Leu Lys Asp Leu Thr Phe Glu Lys Val Lys His Asn Leu Val Ile>
 ____b____b____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b____b____b____>

2410 2420 2430 2440
 * * * * * *
 ACG AAT AGC AAA AAA GAG AAA GTG ACC ATT CAA AAC TGG TTC CGA GAG
 TGC TTA TCG TTT TTT CTC TTT CAC TGG TAA GTT TTG ACC AAG GCT CTC
 Thr Asn Ser Lys Lys Glu Lys Val Thr Ile Gln Asn Trp Phe Arg Glu>
 ____b____b____RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b____b____b____>

Figure 10-8

2450 2460 2470 2480 2490
 * * * * * * *
 GCT GAT TTT GCT AAA GAA GTG CCT AAT TAT AAA GCA ACT AAA GAT GAG
 CGA CTA AAA CGA TTT CTT CAC GGA TTA ATA TTT CGT TGA TTT CTA CTC
 Ala Asp Phe Ala Lys Glu Val Pro Asn Tyr Lys Ala Thr Lys Asp Glu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2500 2510 2520 2530 2540
 * * * * * * *
 AAA ATC GAA GAA ATC ATC GGT CAA AAT GGC GAG CGG ATC ACC TCA AAG
 TTT TAG CTT CTT TAG TAG CCA GTT TTA CCG CTC GCC TAG TGG AGT TTC
 Lys Ile Glu Glu Ile Ile Gly Gln Asn Gly Glu Arg Ile Thr Ser Lys>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2550 2560 2570 2580 2590
 * * * * * * *
 CAA GTT GAT GAT CTT ATC GCA AAA GGT AAC GGC AAA ATT ACC CAA GAT
 GTT CAA CTA CTA GAA TAG CGT TTT CCA TTG CCG TTT TAA TGG GTT CTA
 Gln Val Asp Asp Leu Ile Ala Lys Gly Asn Gly Lys Ile Thr Gln Asp>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2600 2610 2620 2630 2640
 * * * * * * *
 GAG CTA TCA AAA GTT GTT GAT AAC TAT GAA TTG CTC AAA CAT AGC AAA
 CTC GAT AGT TTT CAA CAA CTA TTG ATA CTT AAC GAG TTT GTA TCG TTT
 Glu Leu Ser Lys Val Val Asp Asn Tyr Glu Leu Leu Lys His Ser Lys>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2650 2660 2670 2680
 * * * * * * *
 AAT GTG ACA AAC AGC TTA GAT AAG TTA ATC TCA TCT GTA AGT GCA TTT
 TTA CAC TGT TTG TCG AAT CTA TTC AAT TAG AGT AGA CAT TCA CGT AAA
 Asn Val Thr Asn Ser Leu Asp Lys Leu Ile Ser Ser Val Ser Ala Phe>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

2690 2700 2710 2720 2730
 * * * * * * *
 ACC TCG TCT AAT GAT TCG AGA AAT GTA TTA GTG GCT CCA ACT TCA ATG
 TGG AGC AGA TTA CTA AGC TCT TTA CAT AAT CAC CGA GGT TGA AGT TAC
 Thr Ser Ser Asn Asp Ser Arg Asn Val Leu Val Ala Pro Thr Ser Met>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

Figure 10-9

Variable	Mean	SD	Min	Max
Age	38.5	12.5	25	65
Gender	0.5	0.5	0	1
Marital Status	0.5	0.5	0	1
Education	12.5	2.5	9	16
Income	3500	1500	1000	8000
Health Status	0.5	0.5	0	1
Exercise Frequency	2.5	1.5	0	5
Stress Level	4.5	1.5	1	7
Sleep Quality	3.5	1.5	1	6
Dietary Habits	0.5	0.5	0	1
Work Hours	40	10	20	60
Family Size	2.5	1.5	1	5
Home Ownership	0.5	0.5	0	1
Travel Frequency	1.5	1.5	0	5
Pet Ownership	0.5	0.5	0	1
Volunteering	0.5	0.5	0	1
Religious Beliefs	0.5	0.5	0	1
Political Views	0.5	0.5	0	1
Artistic Interests	0.5	0.5	0	1
Gardening	0.5	0.5	0	1
Reading Habits	0.5	0.5	0	1
Music Interests	0.5	0.5	0	1
Video Gaming	0.5	0.5	0	1
Collecting	0.5	0.5	0	1
Traveling	0.5	0.5	0	1
Volunteering	0.5	0.5	0	1
Religious Beliefs	0.5	0.5	0	1
Political Views	0.5	0.5	0	1
Artistic Interests	0.5	0.5	0	1
Gardening	0.5	0.5	0	1
Reading Habits	0.5	0.5	0	1
Music Interests	0.5	0.5	0	1
Video Gaming	0.5	0.5	0	1
Collecting	0.5	0.5	0	1
Traveling	0.5	0.5	0	1

Variable	Mean	SD	Min	Max
Age	34.5	10.2	21	55
Gender	0.5	0.5	0	1
Marital status	0.6	0.5	0	1
Education	12.5	1.5	9	16
Income	1500	500	500	3000
Health status	0.8	0.2	0	1
Smoking status	0.3	0.5	0	1
Alcohol consumption	0.2	0.4	0	1
Exercise frequency	0.5	0.5	0	1
Stress level	0.7	0.3	0	1
Sleep quality	0.6	0.4	0	1
Work satisfaction	0.5	0.5	0	1
Life satisfaction	0.6	0.4	0	1
Depression score	10.5	5.0	0	30
Anxiety score	12.0	6.0	0	30
Quality of life score	75.0	10.0	50	100

Variable	Mean	SD	Min	Max
Age	38.5	12.5	25	65
Gender	0.5	0.5	0	1
Marital Status	0.7	0.5	0	1
Education	12.5	2.5	9	16
Income	1500	500	500	3000
Health Status	0.8	0.4	0	1
Exercise Frequency	2.5	1.5	0	5
Stress Level	3.5	1.5	1	5
Sleep Quality	4.0	1.0	3	5
Dietary Habits	3.0	1.0	2	4
Work-Life Balance	3.5	1.0	2	4
Family Support	4.5	1.0	3	5
Community Involvement	2.0	1.0	1	3
Overall Well-being	4.0	1.0	3	5

Variable	Mean	SD	Min	Max
Age	34.5	10.2	21	55
Gender	0.5	0.5	0	1
Marital status	0.6	0.5	0	1
Education	12.5	1.5	9	16
Income	1500	500	500	3000
Health status	0.8	0.2	0	1
Smoking status	0.3	0.5	0	1
Alcohol consumption	0.2	0.4	0	1
Exercise frequency	0.5	0.5	0	1
Stress level	0.7	0.3	0	1
Depression score	0.4	0.5	0	1
Life satisfaction	0.6	0.5	0	1
Quality of life	0.7	0.4	0	1
Healthcare utilization	0.5	0.5	0	1
Health insurance status	0.9	0.1	0	1
Healthcare access	0.8	0.2	0	1
Healthcare cost	1000	300	500	2000
Healthcare quality	0.7	0.3	0	1
Healthcare satisfaction	0.6	0.4	0	1
Healthcare accessibility	0.8	0.2	0	1
Healthcare affordability	0.7	0.3	0	1
Healthcare effectiveness	0.6	0.4	0	1
Healthcare safety	0.8	0.2	0	1
Healthcare equity	0.7	0.3	0	1
Healthcare transparency	0.6	0.4	0	1
Healthcare accountability	0.7	0.3	0	1
Healthcare integrity	0.8	0.2	0	1
Healthcare honesty	0.7	0.3	0	1
Healthcare trustworthiness	0.6	0.4	0	1
Healthcare reliability	0.7	0.3	0	1
Healthcare predictability	0.8	0.2	0	1
Healthcare consistency	0.7	0.3	0	1
Healthcare stability	0.8	0.2	0	1
Healthcare durability	0.7	0.3	0	1
Healthcare longevity	0.8	0.2	0	1
Healthcare sustainability	0.7	0.3	0	1
Healthcare viability	0.8	0.2	0	1
Healthcare feasibility	0.7	0.3	0	1
Healthcare practicality	0.8	0.2	0	1
Healthcare applicability	0.7	0.3	0	1
Healthcare adaptability	0.8	0.2	0	1
Healthcare flexibility	0.7	0.3	0	1
Healthcare scalability	0.8	0.2	0	1
Healthcare portability	0.7	0.3	0	1
Healthcare interoperability	0.8	0.2	0	1
Healthcare compatibility	0.7	0.3	0	1
Healthcare coherence	0.8	0.2	0	1
Healthcare consistency	0.7	0.3	0	1
Healthcare homogeneity	0.8	0.2	0	1
Healthcare uniformity	0.7	0.3	0	1
Healthcare regularity	0.8	0.2	0	1
Healthcare predictability	0.7	0.3	0	1
Healthcare stability	0.8	0.2	0	1
Healthcare durability	0.7	0.3	0	1
Healthcare longevity	0.8	0.2	0	1
Healthcare sustainability	0.7	0.3	0	1
Healthcare viability	0.8	0.2	0	1
Healthcare feasibility	0.7	0.3	0	1
Healthcare practicality	0.8	0.2	0	1
Healthcare applicability	0.7	0.3	0	1
Healthcare adaptability	0.8	0.2	0	1
Healthcare flexibility	0.7	0.3	0	1
Healthcare scalability	0.8	0.2	0	1
Healthcare portability	0.7	0.3	0	1
Healthcare interoperability	0.8	0.2	0	1
Healthcare compatibility	0.7	0.3	0	1
Healthcare coherence	0.8	0.2	0	1
Healthcare consistency	0.7	0.3	0	1
Healthcare homogeneity	0.8	0.2	0	1
Healthcare uniformity	0.7	0.3	0	1
Healthcare regularity	0.8	0.2	0	1
Healthcare predictability	0.7	0.3	0	1
Healthcare stability	0.8	0.2	0	1
Healthcare durability	0.7	0.3	0	1
Healthcare longevity	0.8	0.2	0	1
Healthcare sustainability	0.7	0.3	0	1
Healthcare viability	0.8	0.2	0	1
Healthcare feasibility	0.7	0.3	0	1
Healthcare practicality	0.8	0.2	0	1
Healthcare applicability	0.7	0.3	0	1
Healthcare adaptability	0.8	0.2	0	1
Healthcare flexibility	0.7	0.3	0	1
Healthcare scalability	0.8	0.2	0	1
Healthcare portability	0.7			

COMBINED DECLARATION AND POWER OF ATTORNEY
FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, I HEREBY DECLARE THAT:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ENHANCED IMMUNOGENICITY USING LEUKOTOXIN CHIMERAS
the specification of which

(check one) x is attached hereto
 was filed on * 19*

as application serial no. * and was amended on * (if applicable).

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I acknowledge and understand that I am an individual who has a duty to disclose information which is material to the patentability of the claims of this application in accordance with Title 37, Code of Federal Regulations, §§ 1.56(a) and (b) which state:

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) It refutes, or is inconsistent with, a position the applicant takes in:

(i) Opposing an argument of unpatentability relied on by the Office, or

(ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

I do not know and do not believe this invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application. This invention was not in public use or on sale in the United States of America more than one year prior to this application. This invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than six months prior to this application.

I hereby appoint the following attorneys and agents to prosecute that application and to transact all business in the Patent and Trademark Office connected therewith and to file, to prosecute and to transact all business in connection with all patent applications directed to the invention:

Dianne E. Reed, Reg. No. 31,292

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Signature: 


Date October 9, 1992

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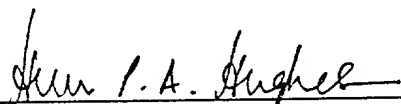
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Atty Dkt 9001-0016.01
PATENT

"Express Mail" Mailing Label No. EM 049 296 147 US
Date of Deposit NOVEMBER 24, 1997

I hereby certify that this paper or fee is being deposited with the United States Postal Service
"Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated
above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

PATRICIA K HIMENES
Typed or Printed Name of Person Mailing Paper or Fee

Patricia K Himenes
Signature of Person Mailing Paper or Fee

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

ANDREW A. POTTER et al.

Serial No.: CON of 08/455,970

Group Art Unit: Unassigned

Filing Date: Even date

Examiner: Unassigned

Title: ENHANCED IMMUNOGENICITY USING LEUKOTOXIN CHIMERAS

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE
WITH 37 C.F.R. §§ 1.821-1.825

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned hereby states that the content of the attached papers and the computer-readable copy of
the Sequence Listing, submitted in accordance with 37 C.F.R. §§ 1.821(c) and (e), respectively, are the same.

Respectfully submitted,

Date: 11/24/97

By: Roberta L. Robins
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089666-1497

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: POTTER, ANDREW A.
REDMOND, MARK J.
HUGHES, HUW P.A.
- (ii) TITLE OF INVENTION: ENHANCED IMMUNOGENICITY USING LEUKOTOXIN CHIMERAS
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: ROBERTA L. ROBINS
 - (B) STREET: 635 BRYANT STREET
 - (C) CITY: PALO ALTO
 - (D) STATE: CALIFORNIA
 - (E) COUNTRY: UNITED STATES OF AMERICA
 - (F) ZIP: 94301
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 07/960,932
 - (B) FILING DATE: 14-OCT-1992
 - (C) CLASSIFICATION: 435
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: ROBINS, ROBERTA L.
 - (B) REGISTRATION NUMBER: 33,208
 - (C) REFERENCE/DOCKET NUMBER: 9000-0016.20
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (415) 617-8999
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2794 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..2778

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGCTACTG TTATAGATCT AAGCTTCCCA AAAACTGGGG CAAAAAAT TATCCTCTAT	60
ATTCGCCAAA ATTACCAATA TGATACTGAA CAAGGTAATG GTTTACAGGA TTTAGTCAAA	120
GCGGCCGAAG AGTTGGGGAT TGAGGTACAA AGAGAAGAAC GCAATAATAT TGCAACAGCT	180

CAAACCAAGTT TAGGCACGAT TCAAACCGCT ATTGGCTTAA CTGACCGTGG CATTGTGTGA	240
TCCGCTCCAC AAATTGATAA ATTGCTACAG AAAACTAAAG CAGGCCAAGC ATTAGGTTCT	300
GCCGAAAGCA TTGTACAAA TGCAATAAA GCCAAACTG TATTATCTGG CATTCAATCT	360
ATTTTAGGCT CAGTATTGGC TGGGAATGGAT TTAGATGAGG CCTTACAGAA TAACAGCAAC	420
CAACATGCTC TTGCTAAAGC TGGCTTGGAG CTAACAAATT CATTAATTGA AAATATTGCT	480
AATTCAGTAA AAACACTTGA CGAATTTGGT GAGCAAATTA GTCAATTTGG TTCAAACTA	540
CAAAATATCA AAGGCTTAGG GACTTTAGGA GACAACTCA AAAATATCGG TGGACTTGAT	600
AAAGCTGGCC TTGGTTTAGA TGTTATCTCA GGGCTATTAT CGGGCGCAAC AGCTGCACTT	660
GTACTTGCAG ATAAAAATGC TTCAACAGCT AAAAAAGTGG GTGCGGGTTT TGAATTGGCA	720
AACCAAGTTG TTGGTAATAT TACCAAAGCC GTTCTTCTT ACATTTTAGC CCAACGTGTT	780
GCAGCAGGTT TATCTTCAAC TGGGCCTGTG GCTGCTTAA TTGCTTCTAC TGTTTCTCTT	840
GCGATTAGCC CATTAGCATT TGCCGGTATT GCCGATAAAT TTAATCATGC AAAAGTTTA	900
GAGAGTTATG CCGAACGCTT TAAAAATTA GGCTATGACG GAGATAATTT ATTAGCAGAA	960
TATCAGCGGG GAACAGGGAC TATTGATGCA TCGGTTACTG CAATTAATAC CGCATTGGCC	1020
GCTATTGCTG GTGCTGTGTC TGCTGCTGCA GCCGGCTCGG TTATTGCTTC ACCGATTGCC	1080
TTATTAGTAT CTGGGATTAC CGGTGTAATT TCTACGATTC TGCAATATTC TAAACAAGCA	1140
ATGTTTGAGC ACGTTGCAA TAAATTCAT AACAAATTG TAGAATGGGA AAAAAATAAT	1200
CACGGTAAGA ACTACTTTGA AAATGGTTAC GATGCCCGTT ATCTTGCGAA TTTACAAGAT	1260
AATATGAAAT TCTTACTGAA CTTAAACAAA GAGTTACAGG CAGAACGTGT CATCGCTATT	1320
ACTCAGCAGC AATGGGATAA CAACATTGGT GATTAGCTG GTATTAGCCG TTTAGGTGAA	1380
AAAGTCCTTA GTGGTAAAGC CTATGTGGAT GCGTTTGAAG AAGGCAAACA CATTAAAGCC	1440
GATAAATTAG TACAGTTGGA TTCGGCAAAC GGTATATTG ATGTGAGTAA TTCGGGTAAA	1500
GCGAAACTC AGCATATCTT ATTCAGAACG CCATTATTGA CGCCGGGAAC AGAGCATCGT	1560
GAACGCGTAC AAACAGGTAA ATATGAATAT ATTACCAAGC TCAATATTAA CCGTGTAGAT	1620
AGCTGGAAAA TTACAGATGG TGCAGCAAGT TCTACCTTTG ATTTAACTAA CGTTGTTTACG	1680
CGTATTGGTA TTGAATTAGA CAATGCTGGA AATGTAAC TAACCAAAGA AACAAAAATT	1740
ATTGCCAAAC TTGGTGAAGG TGATGACAAC GTATTTGTTG GTTCTGGTAC GACGGAAATT	1800
GATGGCGGTG AAGGTTACGA CCGAGTTCAC TATAGCCGTG GAACTATGG TGCTTTAACT	1860
ATTGATGCAA CCAAGAGAC CGAGCAAGGT AGTTATACCG TAAATCGTTT CGTAGAAACC	1920
GGTAAAGCAC TACACGAAGT GACTTCAACC CATACCGCAT TAGTGGGCAA CCGTGAAGAA	1980
AAAATAGAAT ATCGTCATAG CAATAACCAG CACCATGCCG GTTATTACAC CAAAGATACC	2040

TTGAAAGCTG TTGAAGAAAT TATCGSTACA TCACATAACG ATATCTTTAA AGGTAGTAAG 2100
 TTCAATGATG CCTTTAACGG TGGTGATGGT GTCGATACTA TTGACGGTAA CGACGGCAAT 2150
 GACCGCTTAT TTGGTGGTAA AGGCGATGAT ATTCTCGATG GTGGAAATGG TGATGATTTT 2220
 ATCGATGGCG GTAAAGGCAA CGACCTATTA CACGGTGGCA AGGGCGATGA TATTTTCGTT 2280
 CACCGTAAAG GCGATGGTAA TGATATTATT ACCGATTCTG ACGGCAATGA TAAATTATCA 2340
 TTCTCTGATT CGAACTTAAA AGATTTAACA TTTGAAAAAG TTAAACATAA TCTTGTCTATC 2400
 ACGAATAGCA AAAAAGAGAA AGTGACCATT CAAAAGTGGT TCCGAGAGGC TGATTTTGCT 2460
 AAAGAAGTGC CTAATTATAA AGCAACTAAA GATGAGAAAA TCGAAGAAAT CATCGGTCAA 2520
 AATGGCGAGC GGATCACCTC AAAGCAAGTT GATGATCTTA TCGCAAAAGG TAACGGCAAA 2580
 ATTACCCAAG ATGAGCTATC AAAAGTTGTT GATAACTATG AATTGCTCAA ACATAGCATA 2640
 AATGTGACAA ACAGCTTAGA TAAGTTAATC TCATCTGTAA GTGCATTTAC CTCGTCTAAT 2700
 GATTCGAGAA ATGTATTAGT GGCTCCAACT TCAATGTTGG ATCAAAGTTT ATCTTCTCTT 2760
 CAATTTGCTA GGGGATCCTA GCTAGCTAGC CATG 2794

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GATCCAGCTC TTCTGCCGGC TGCAAAACT TCTTCTGGAA AACCTTCACC AGCTGCTAGG 60

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATCCCTAGC AGCTGGTGAA GGTTTTCCAG AAGAAGTTTT TGCAGCCGGC AGAAGAGCTG 60

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 39 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GATCTCAGCA TTGGAGCTAC GGCCTGCGCC CTGGCTAAG

39

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATCCTTAGC CAGGGCGCAG GCCGTAGCTC CAATGCTGA

39

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 83 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GATCTTGCAA CATTGTGCCT GTGAGCATTG TGAGCCGCAA CATTGTGTAC ACCCGCGCGC

60

AACCTAACCA AGACATTGTG TAG

83

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 83 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATCCTACAC AATGTCTTGG TTAAGTTGCG CGCGGGTGTA CACAATGTTG CGGCTCACAA

60

TCGTCACAGG CACAATGTTG CAA

83

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2838 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..2829

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGGCTACTG TTATAGATCT AAGCTTCCCA AAAACTGGGG CAAAAAAAT TATCCTCTAT	60
ATTCCCCAAA ATTACCAATA TGATACTGAA CAAGGTAATG GTTTACAGGA TTTAGTCAAA	120
GCGGCCGAAG AGTTGGGGAT TGAGGTACAA AGAGAAGAAC GCAATAATAT TGCAACAGCT	180
CAAACAGTT TAGGCACGAT TCAAACCGCT ATTGGCTTAA CTGAGCGTGG CATTGTGTTA	240
TCCGCTCCAC AAATTGATAA ATTGCTACAG AAAACTAAAG CAGGCCAAGC ATTAGGTTCT	300
GCCGAAAGCA TTGTACAAAA TGCAATATA GCCAAAACCTG TATTATCTGG CATTCAATCT	360
ATTTTAGGCT CAGTATTGGC TGGAAATGGAT TTAGATGAGG CCTTACAGAA TAACAGCAAC	420
CAACATGCTC TTGCTAAAGC TGGCTTGGAG CTAACAAATT CATTAATTGA AAATATTGCT	480
AATTCAGTAA AAACACTTGA CGAATTTGGT GACCAAATTA GTCAATTTGG TTCAAAATA	540
CAAAATATCA AAGGCTTAGG GACTTTAGGA GACAACTCA AAAATATCGG TGGACTTGAT	600
AAAGCTGGCC TTGGTTTAGA TGTTATCTCA GGGCTATTAT CGGGCGCAAC AGCTGCACTT	660
GTACTTGCAAG ATAAAAATGC TTCAACAGCT AAAAAGTGG GTGCGGGTTT TGAATTGGCA	720
AACCAAGTTG TTGGTAATAT TACCAAGCC GTTCTTCTT ACATTTTAGC CCAACGTGTT	780
GCAGCAGGTT TATCTTCAAC TGGGCCTGTG GCTGCTTTAA TTGCTTCTAC TGTCTCTCTT	840
GCGATTAGCC CATTAGCATT TGCCGGTATT GCCGATAAAT TTAATCATGC AAAAGTTTA	900
GAGAGTTATG CCGAACGCTT TAAAAATTA GGCTATGACG GAGATAATTT ATTAGCAGAA	960
TATCAGCGGG GAACAGGGAC TATTGATGCA TCGTTACTG CAATTAATAC CGCATTGGCC	1020
GCTATTGCTG GTGGTGTGTC TGCTGCTGCA GCCGGCTCGG TTATTGCTTC ACCGATTGCC	1080
TTATTAGTAT CTGGGATTAC CGGTGTAAT TCTACGATTG TGCAATATTC TAAACAAGCA	1140
ATGTTTGAGC ACGTTGCAAA TAAATTCAT AACAAAATTG TAGAATGGGA AAAAAATAAT	1200
CACGGTAAGA ACTACTTTGA AAATGGTTAC GATGCCCCGT ATCTTGCGAA TTTACAAGAT	1260
AATATGAAAT TCTTACTGAA CTTAAACAAA GAGTTACAGG CAGAACGTGT CATCGCTATT	1320
ACTCAGCAGC AATGGGATAA CAACATTGGT GATTTAGCTG GTATTAGCCG TTTAGGTGAA	1380
AAAGTCCTTA GTGGTAAAGC CTATGTGGAT GCGTTTGAAG AAGGCAAACA CATTAAAGCC	1440
GATAAATTAG TACAGTTGGA TTCGGCAAAC GGTATTATTG ATGTGAGTAA TTCGGGTAAA	1500
GCGAAAACTC AGCATATCTT ATTCAGAACG CCATTATTGA CGCCGGGAAC AGAGCATCGT	1560
GAACGCGTAC AAACAGGTAA ATATGAATAT ATTACCAAGC TCAATATTAA CCGTGTAGAT	1620
AGCTGGAAAA TTACAGATGG TGCAGCAAGT TCTACCTTTG ATTTAACTAA CGTTGTTTCTAG	1680

CGTATTGGTA TTGAATTAGA CAATGCTGGA AATGTAACTA AAACCAAAGA AACAAAAATT 1740
ATTGCCAAAC TTGGTGAAGG TGATGACAAC GTATTTGTTG GTTCTGGTAC GACGGAAATT 1800
GATGGCGGTG AAGGTTACGA CCGAGTTCAC TATAGCCGTG GAAACTATGG TGCTTTAACT 1860
ATTGATGCAA CCAAAGAGAC CGAGCAAGGT AGTTATACCG TAAATCGTTT CGTAGAAACC 1920
GGTAAAGCAC TACACGAAGT GACTTCAACC CATACCGCAT TAGTGGGCAA CCGTGAAGAA 1980
AAAATAGAAT ATCGTCATAG CAATAACCAG CACCATGCCG GTTATTACAC CAAAGATACC 2040
TTGAAAGCTG TTGAAGAAAT TATCGGTACA TCACATAACG ATATCTTTAA AGGTASTAAG 2100
TTCAATGATG CCTTTAACGG TGGTGATGGT GTCGATACTA TTGACGGTAA CGACGGCAAT 2160
GACCGCTTAT TTGGTGGTAA AGGCGATGAT ATTCTCGATG GTGGAAATGG TGATGATTTT 2220
ATCGATGGCG GTAAAGGCAA CGACCTATTA CACGGTGGCA AGGGCGATGA TATTTTCGTT 2280
CACCGTAAAG GCGATGGTAA TGATATTATT ACCGATTCTG ACGGCAATGA TAAATTATCA 2340
TTCTCTGATT CGAACTTAAA AGATTTAACA TTTGAAAAAG TTAAACATAA TCTTGTGATC 2400
ACGAATAGCA AAAAAGAGAA AGTGACCATT CAAACTGGT TCCGAGAGGC TGATTTTGCT 2460
AAAGAAGTGC CTAATTATAA AGCAACTAAA GATGAGAAAA TCGAAGAAAT CATCGGTCAA 2520
AATGGCGAGC GGATCACCTC AAAGCAAGTT GATGATCTTA TCGCAAAAGG TAACGGCAAA 2580
ATTACCCAAG ATGAGCTATC AAAAGTTGTT GATAACTATG AATTGCTCAA ACATAGCAAA 2640
AATGTGACAA ACAGCTTAGA TAAGTTAATC TCATCTGTAA GTGCATTTAC CTCGTCTAAT 2700
GATTTCAGAA ATGIATTAGT GGCTCCAAC TCAATGTTGG ATCAAAGTTT ATCTTCTCTT 2760
CAATTTGCTA GGGGATCCAG CTCTTCTGCC GGCTGCAAAA ACTTCTTCTG GAAAACCTTC 2820
ACCAGCTGCT AGGGATCC 2838

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2817 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..2808

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGGCTACTG TTATAGATCT AAGCTTCCCA AAAACTGGGG CAAAAAAAT TATCCTCTAT 60
ATTCCCCAAA ATTACCAATA TGATACTGAA CAAGGTAATG GTTTACAGGA TTTAGTCAAA 120
GCGGCCGAAG AGTTGGGGAT TGAGGTACAA AGAGAAGAAC GCAATAATAT TGCAACAGCT 180

CAAACCAAGTT TAGGCACGAT TCAAACCGCT ATTGGCTTAA CTGAGCGTGG CATTGTGTTA	240
TCCGCTCCAC AAATTGATAA ATTGCTACAG AAACTAAAG CAGGCCAAGC ATTAGGTTCT	300
GCCGAAAGCA TTGTACAAA TGCAATAAA GCCAAACTG TATTATCTGG CATTCAATCT	360
ATTTTAGGCT CAGTATTGGC TGGAAATGGAT TTAGATGAGG CCTTACAGAA TAACAGCAAC	420
CAACATGCTC TTGCTAAAGC TGGCTTGGAG CTAACAAATT CATTAATTGA AAATATTGCT	480
AATTCAGTAA AAACACTTGA CGAATTTGGT GAGCAAATTA GTCAATTTGG TTCAAACTA	540
CAAAATATCA AAGGCTTAGG GACTTTAGGA GACAACTCA AAAATATCGG TGGACTTGAT	600
AAAGCTGGCC TTGGTTTAGA TGTTATCTCA GGGCTATTAT CGGGCGCAAC AGCTGCACTT	660
GTACTTGCAAG ATAAAAATGC TTCAACAGCT AAAAAAGTGG GTGCGGGTTT TGAATTGGCA	720
AACCAAGTTG TTGGTAATAT TACCAAAGCC GTTTCTTCTT ACATTTTAGC CCAACGTGTT	780
GCAGCAGGTT TATCTTCAAC TGGGCCTGTG GCTGCTTTAA TTGCTTCTAC TGTTTCTCTT	840
GCGATTAGCC CATTAGCATT TGCCGGTATT GCCGATAAAT TTAATCATGC AAAAAGTTTA	900
GAGAGTTATG CCGAACGCTT TAAAAATTA GGCTATGACG GAGATAATTT ATTAGCAGAA	960
TATCAGCGGG GAACAGGGAC TATTGATGCA TCGGTTACTG CAATTAATAC CGCATTGGCC	1020
GCTATTGCTG GTGGTGTGTC TGCTGCTGCA GCCGGCTCGG TTATTGCTTC ACCGATTGCC	1080
TTATTAGTAT CTGGGATTAC CGGTGTAATT TCTACGATTC TGCAATATTC TAAACAAGCA	1140
ATGTTTGAGC ACGTTGCAA TAAATTCAT AACAAATTG TAGAATGGGA AAAAATAAT	1200
CACGGTAAGA ACTACTTTGA AAATGGTTAC GATGCCCGTT ATCTTGCGAA TTTACAAGAT	1260
AATATGAAAT TCTTACTGAA CTTAAACAAA GAGTTACAGG CAGAACGTGT CATCGCTATT	1320
ACTCAGCAGC AATGGGATAA CAACATTGGT GATTTAGCTG GTATTAGCCG TTTAGGTGAA	1380
AAAGTCCTTA GTGGTAAGC CTATGTGGAT GCGTTTGAAG AAGGCAAACA CATTAAAGCC	1440
GATAAATTAG TACAGTTGGA TTCGGCAAAC GGTATTATTG ATGTGAGTAA TTCGGGTAAA	1500
GCGAAAACTC AGCATATCTT ATTGAGAAGC CCATTATTGA CGCCGGGAAC AGAGCATCGT	1560
GAACGCGTAC AAACAGGTAA ATATGAATAT ATTACCAAGC TCAATATTAA CCGTGTAGAT	1620
AGCTGGAAAA TTACAGATGG TGCAGCAAGT TCTACCTTTG ATTTAACTAA CGTTGTTTACG	1680
CGTATTGGTA TTGAATTAGA CAATGCTGGA AATGTAATA AAACCAAAGA AACAAAAATT	1740
ATTGCCAAAC TTGGTGAAGG TGATGACAAC GTATTTGTTG GTTCTGGTAC GACGGAAATT	1800
GATGGCGGTG AAGGTTACGA CCGAGTTCAC TATAGCCGTG GAAACTATGG TGCTTTAACT	1860
ATTGATGCAA CCAAGAGAC CGAGCAAGT AGTTATACCG TAAATCGTTT CGTAGAAACC	1920
GGTAAAGCAC TACACGAAGT GACTTCAACC CATACCGCAT TAGTGGGCAA CCGTGAAGAA	1980
AAAATAGAAT ATCGTCATAG CAATAACCAG CACCATGCCG GTTATTACAC CAAAGATACC	2040

TTGAAAGCTG TTGAAGAAAT TATCGGTACA TCACATAACG ATATCTTTAA AGGTAGTAAG 2100
 TTCAATGATG CCTTTAACGG TGGTGATGGT GTCGATACTA TTGACGGTAA CGACGGCAAT 2160
 GACCGCTTAT TTGGTGGTAA AGGCGATGAT ATTCTCGATG GTGGAAATGG TGATGATTTT 2220
 ATCGATGGCG GTAAAGGCAA CGACCTATTA CACGGTGGCA AGGSCGATGA TATTTTCGTT 2280
 CACCGTAAAG GCGATGGTAA TGATATTATT ACCGATTCTG ACGGCAATGA TAAATTATCA 2340
 TTCTCTGATT CGAACTTAAA AGATTTAACA TTTGAAAAG TTAACATAA TCTTGTCATC 2400
 ACCAATAGCA AAAAAGAGAA AGTGACCATT CAAACTGGT TCCGAGAGGC TGATTTTGCT 2460
 AAAGAAGTGC CTAATTATAA AGCAACTAAA GATGAGAAAA TCGAAGAAAT CATCGGTCAA 2520
 AATGGCGAGC GGATCACCTC AAAGCAAGTT GATGATCTTA TCGCAAAGG TAACGGCAAA 2580
 ATTACCCAAG ATGAGCTATC AAAAGTTGTT GATAACTATG AATTGCTCAA ACATAGCAAA 2640
 AATGTGACAA ACAGCTTAGA TAAGTTAATC TCATCTGTAA GTGCATTTAC CTCGTCTAAT 2700
 GATTCGAGAA ATGTATTAGT GGCTCCAAC TCAATGTTGG ATCAAAGTTT ATCTTCTCTT 2760
 CAATTTGCTA GGGGATCTCA GCATTGGAGC TACGGCCTGC GCCCTGGCTA AGGATCC 2817

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2861 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..2853

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATGGCTACTG TTATAGATCT AAGCTTCCCA AAAACTGGGG CAAAAAAAT TATCCTCTAT 60
 ATTCCCCAAA ATTACCAATA TGATACTGAA CAAGGTAATG GTTTACAGGA TTTAGTCAA 120
 GCGGCCGAAG AGTTGGGGAT TGAGGTACAA AGAGAAGAAC GCAATAATAT TGCAACAGCT 180
 CAAACCAGTT TAGGCACGAT TCAAACCGCT ATTGGCTTAA CTGAGCGTGG CATTGTGTTA 240
 TCCGCTCCAC AAATTGATAA ATTGCTACAG AAAACTAAAG CAGGCCAAGC ATTAGGTTCT 300
 GCCGAAAGCA TTGTACAAAA TGCAAATAAA GCCAAACTG TATTATCTGG CATTCAATCT 360
 ATTTTAGGCT CAGTATTGGC TGGAAATGGAT TTAGATGAGG CCTTACAGAA TAACAGCAAC 420
 CAACATGCTC TTGCTAAAGC TGGCTTGGAG CTAACAAATT CATTAATTGA AAATATTGCT 480
 AATTCAGTAA AAACACTTGA CGAATTTGGT GAGCAAATTA GTCAATTTGG TTCAAAATA 540
 CAAAATATCA AAGGCTTAGG GACTTTAGGA GACAACTCA AAAATATCGG TGGACTTGAT 600

AAAGCTGGCC TTGGTTTAGA TGTTATCTCA GGGCTATTAT CGGGCGCAAC AGCTGCACTT	660
GTACTTGACAG ATAAAAATGC TTCAACAGCT AAAAAAGTGG GTGCGGGTTT TGAATTGGCA	720
AACCAAGTTG TTGGTAATAT TACCAAAGCC GTTCTTCTT ACATTTTAGC CCAACGTGTT	780
GCAGCAGGTT TATCTTCAAC TGGGCCTGTG GCTGCTTTAA TTGCTTCTAC TGTCTCTCTT	840
GCGATTAGCC CATTAGCATT TGCCGGTATT GCCGATAAAT TTAATCATGC AAAAAGTTTA	900
GAGAGTTATG CCGAACGCTT TAAAAATTA GGCTATGACG GAGATAATTT ATTAGCAGAA	960
TATCAGCGGG GAACAGGSAC TATTGATGCA TCGGTACTG CAATTAATAC CGCATTGGCC	1020
GCTATTGCTG GTGGTGTGTC TGCTGCTGCA GCCGCTCGG TTATTGCTTC ACCGATTGCC	1080
TTATTAGTAT CTGGGATTAC CGGTGTAATT TCTACGATTC TGCAATATTC TAAACAAGCA	1140
ATGTTTGAGC ACGTTGCAA TAAATTCAT AACAAAATTG TAGAATGGGA AAAAAATAAT	1200
CACGGTAAGA ACTACTTTGA AAATGGTTCAT GATGCCCGTT ATCTTGCGAA TTTACAAGAT	1260
AATATGAAAT TCTTACTGAA CTTAAACAAA GAGTTACAGG CAGAACGTGT CATCGCTATT	1320
ACTCAGCAGC AATGGGATAA CAACATTGGT GATTAGCTG GTATTAGCCG TTTAGGTCAA	1380
AAAGTCCTTA GTGGTAAAGC CTATGTGGAT GCGTTTGAAG AAGGCAAACA CATTAAAGCC	1440
GATAAATTAG TACAGTTGGA TTCGGCAAAC GGTATTATTG ATGTGAGTAA TTCGGGTAAA	1500
GCGAAACTC AGCATATCTT ATTCAGAACG CCATTATTGA CGCCGGGAAC AGAGCATCGT	1560
GAACGCGTAC AAACAGGTAA ATATGAATAT ATTACCAAGC TCAATATTAA CCGTGTAGAT	1620
AGCTGGAAAA TTACAGATGG TGCAGCAAGT TCTACCTTTG ATTTAACTAA CGTTGTTTCTAG	1680
CGTATTGGTA TTGAATTAGA CAATGCTGGA AATGTAAC TAACCAAAGA AACAAAAATT	1740
ATTGCCAAAC TTGGTGAAGG TGATGACAAC GTATTGTTG GTTCTGGTAC GACGGAAATT	1800
GATGGCGGTG AAGGTTACGA CCGAGTTCAC TATAGCCGTG GAAACTATGG TGCTTTAACT	1860
ATTGATGCAA CCAAAGAGAC CGAGCAAGGT AGTTATACCG TAAATCGTTT CGTAGAAACC	1920
GGTAAAGCAC TACACGAAGT GACTTCAACC CATACCGCAT TAGTGGGCAA CCGTGAAGAA	1980
AAAATAGAAT ATCGTCATAG CAATAACCAG CACCATGCCG GTTATTACAC CALAGATACC	2040
TTGAAAGCTG TTGAAGAAAT TATCGGTACA TCACATAACG ATATCTTTAA AGGTAGTAAG	2100
TTCAATGATG CCTTTAACGG TGGTGATGGT GTCGATACTA TTGACGGTAA CGACGGCAAT	2160
GACCGCTTAT TTGGTGGTAA AGGCGATGAT ATTCTCGATG GTGGAAATGG TGATGATTTT	2220
ATCGATGGCG GTAAAGGCAA CGACCTATTA CACGGTGGCA AGGGCGATGA TATTTTCGTT	2280
CACCGTAAAG GCGATGGTAA TGATATTATT ACCGATTCTG ACGGCAATGA TAAATTATCA	2340
TTCTCTGATT CGAACTTAAA AGATTTAACA TTTGAAAAAG TTAAACATAA TCTTGTCTATC	2400
ACGAATAGCA AAAAAGAGAA AGTGACCATT CAAAACCTGGT TCCGAGAGGC TGATTTTGCT	2460

AAAGAAGTGC CTAATTATAA AGCAACTAAA GATGAGAAAA TCGAAGAAAT CATCGGTCAA 2520
AATGGCGAGC GGATCACCTC AAAGCAAGTT GATGATCTTA TCGCAAPAGG TAACGGCAAA 2580
ATTACCCAAG ATGAGCTATC AAAAGTTGTT GATAACTATG AATTGCTCAA ACATAGCAAA 2640
AATGTGACAA ACAGCTTAGA TAAGTTAATC TCATCTGTAA GTGCATTAC CTCGTCTAAT 2700
GATTCGAGAA ATGTATTAGT GGCTCCAAC TCAATGTTGG ATCAAAGTTT ATCTTCTCTT 2760
CAATTTGCTA GGGGATCTTG CAACATTGTG CCTGTGAGCA TTGTGAGCCG CAACATTGTG 2820
TACACCCGCG CGCAACCTAA CCAAGACATT GTGTAGGATC C 2861

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 3
 - (D) OTHER INFORMATION: /note= "The amino acid at this location can be either Lys, Asp, Val or Asn."
- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 5
 - (D) OTHER INFORMATION: /note= "The amino acid at this location can be either Lys, Asp, Val or Asn."
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Gly Xaa Gly Xaa Asp
1 5

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

PATRICIA K. HIMENES
Typed or Printed Name of Person Mailing Paper or Fee
Patricia K. Himenes
Signature of Person Mailing Paper or Fee

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

ANDREW A. POTTER et al.

Serial No.: CON of 08/455,970

Group Art Unit: Unassigned

Filing Date: On Even Date Herewith

Examiner: Unassigned

Title: ENCHANCED IMMUNOGENICITY USING LEUKOTOXIN
CHIMERAS

SUBMISSION OF FORMAL DRAWINGS

Assistant Commissioner for Patents
Washington, D.C. 20231

Attention: Official Draftsman

Sir:

Enclosed are 45 sheet(s) of formal drawings in connection with the above identified case.

Respectfully submitted,

Date: 11/24/97

By: Roberta L. Robins

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Registration No. 33,208

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0897636-1.449

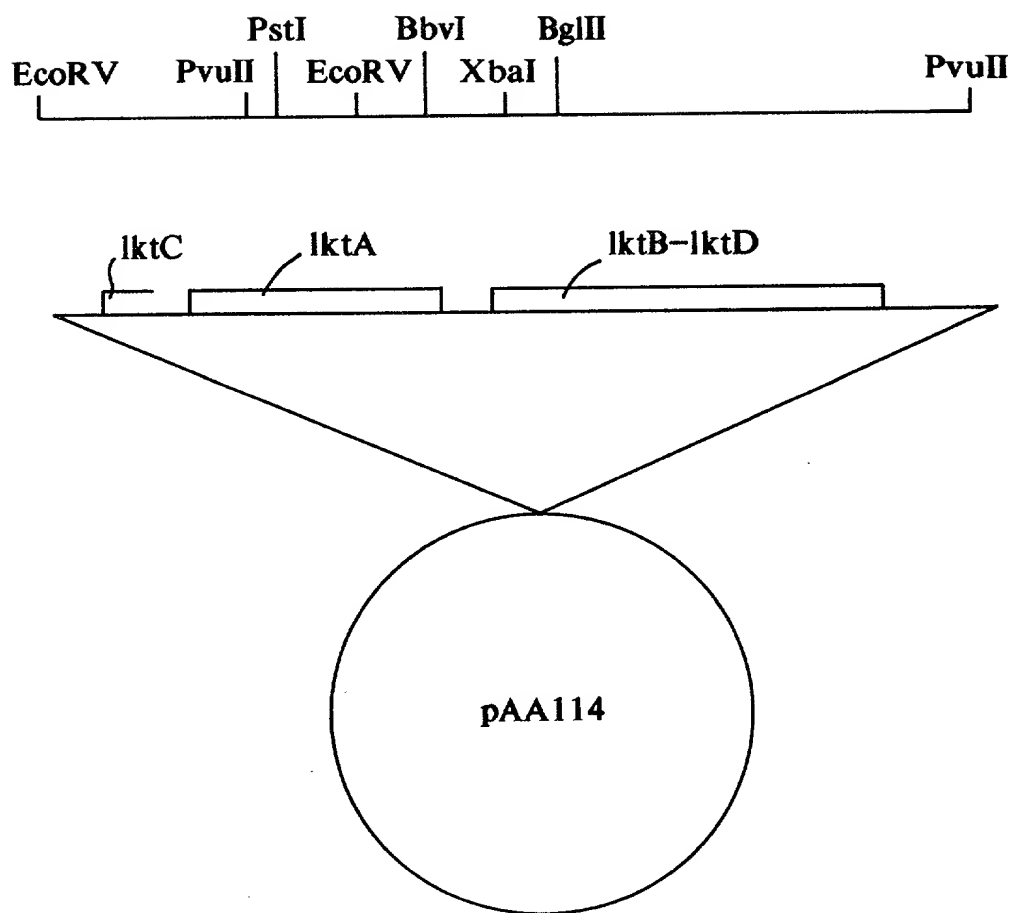


FIG. 1

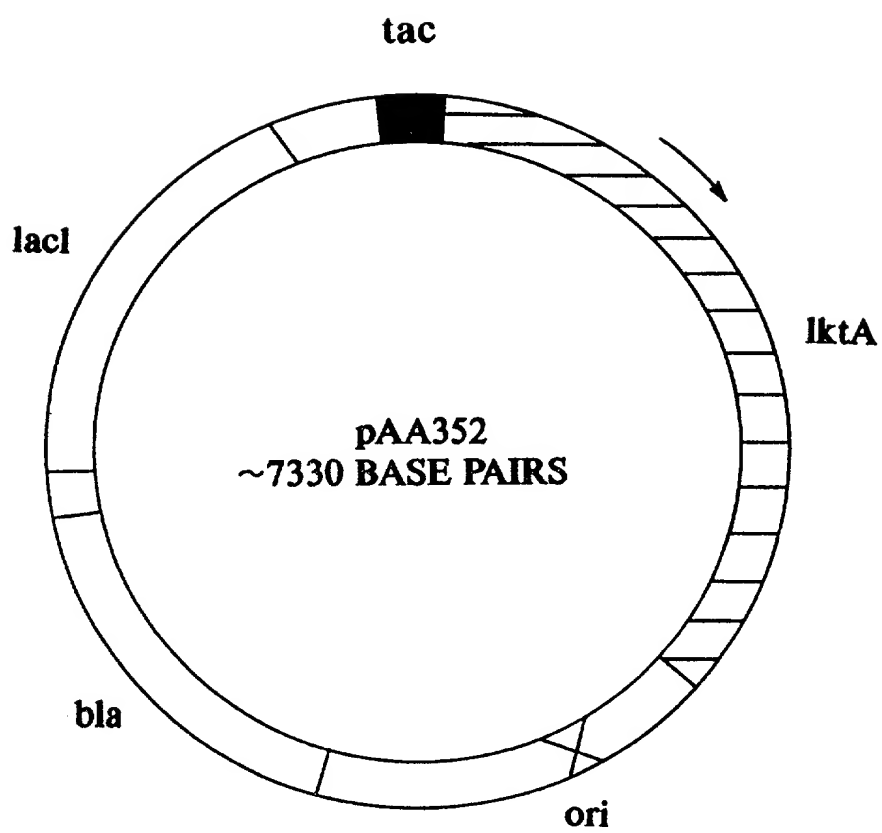


FIG. 2

SRIF-1: 5'-GATCCAGCTCTTCTGCCGGCTGCAAAACTTCTTCTGGAACCTTCACCAGCTGCTAGG-3'
SRIF-2: 3'-GTCGAGAGAGACGGCCGACGTTTTTTGAAGAGACCCTTTGGAGTGGTCGACGATCCCTAG-5'

GNRH-1: 5'-GATCTCAGCATTGGAAGTACGGGCTGCGCCCTGGCTAAG-3'
GNRH-2: 3'-AGTCGTAACTTCGATGCCGGACGCGGGACCGATTCTAG-5'

VP4-1: 5'-GATCTTGCAACATTGTGCTGTGAGCATTGTGAGCCGCAACATTGTGTACACCCGGCGCAACCTAACCAAGACATTGTGTAG-3'
VP4-2: 3'-AACGTTGTAACACGGACACTCGTAACACTCGGCCGTTGTAAACACATGTGGGCGCGGTTGAATTGGTTCTGTAAACACATCCTAG-5'

FIG. 4

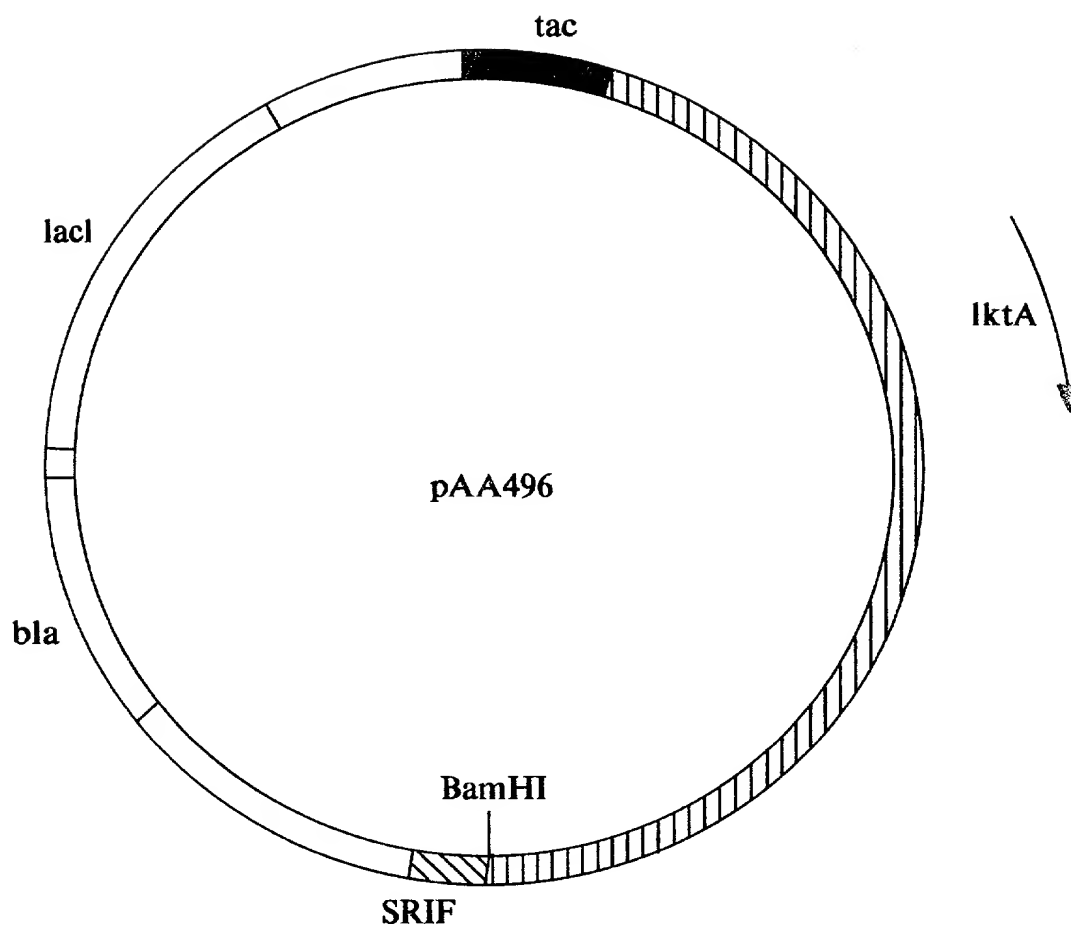


FIG. 5

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      10      20      30      40
      *      *      *      *      *      *
ATG GCT ACT GTT ATA GAT CTA AGC TTC CCA AAA ACT GGG GCA AAA AAA
TAC CGA TGA CAA TAT CTA GAT TCG AAG GGT TTT TGA CCC CGT TTT TTT
Met Ala Thr Val Ile Asp Leu Ser Phe Pro Lys Thr Gly Ala Lys Lys>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

50      60      70      80      90
      *      *      *      *      *      *
ATT ATC CTC TAT ATT CCC CAA AAT TAC CAA TAT GAT ACT GAA CAA GGT
TAA TAG GAG ATA TAA GGG GTT TTA ATG GTT ATA CTA TGA CTT GTT CCA
Ile Ile Leu Tyr Ile Pro Gln Asn Tyr Gln Tyr Asp Thr Glu Gln Gly>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

100      110      120      130      140
      *      *      *      *      *      *
AAT GGT TTA CAG GAT TTA GTC AAA GCG GCC GAA GAG TTG GGG ATT GAG
TTA CCA AAT GTC CTA AAT CAG TTT CGC CGG CTT CTC AAC CCC TAA CTC
Asn Gly Leu Gln Asp Leu Val Lys Ala Ala Glu Glu Leu Gly Ile Glu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

150      160      170      180      190
      *      *      *      *      *      *
GTA CAA AGA GAA GAA CGC AAT AAT ATT GCA ACA GCT CAA ACC AGT TTA
CAT GTT TCT CTT CTT GCG TTA TTA TAA CGT TGT CGA GTT TGG TCA AAT
Val Gln Arg Glu Glu Arg Asn Asn Ile Ala Thr Ala Gln Thr Ser Leu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

200      210      220      230      240
      *      *      *      *      *      *
GGC ACG ATT CAA ACC GCT ATT GGC TTA ACT GAG CGT GGC ATT GTG TTA
CCG TGC TAA GTT TGG CGA TAA CCG AAT TGA CTC GCA CCG TAA CAC AAT
Gly Thr Ile Gln Thr Ala Ile Gly Leu Thr Glu Arg Gly Ile Val Leu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

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FIG. 6A

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      250      260      270      280
      *      *      *      *      *      *      *      *
TCC GCT CCA CAA ATT GAT AAA TTG CTA CAG AAA ACT AAA GCA GGC CAA
AGG CGA GGT GTT TAA CTA TTT AAC GAT GTC TTT TGA TTT CGT CCG GTT
Ser Ala Pro Gln Ile Asp Lys Leu Leu Gln Lys Thr Lys Ala Gly Gln>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

290      300      310      320      330
      *      *      *      *      *      *      *      *
GCA TTA GGT TCT GCC GAA AGC ATT GTA CAA AAT GCA AAT AAA GCC AAA
CGT AAT CCA AGA CGG CTT TCG TAA CAT GTT TTA CGT TTA TTT CGG TTT
Ala Leu Gly Ser Ala Glu Ser Ile Val Gln Asn Ala Asn Lys Ala Lys>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      340      350      360      370      380
      *      *      *      *      *      *      *      *
ACT GTA TTA TCT GGC ATT CAA TCT ATT TTA GGC TCA GTA TTG GCT GGA
TGA CAT AAT AGA CCG TAA GTT AGA TAA AAT CCG AGT CAT AAC CGA CCT
Thr Val Leu Ser Gly Ile Gln Ser Ile Leu Gly Ser Val Leu Ala Gly>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      390      400      410      420      430
      *      *      *      *      *      *      *      *
ATG GAT TTA GAT GAG GCC TTA CAG AAT AAC AGC AAC CAA CAT GCT CTT
TAC CTA AAT CTA CTC CGG AAT GTC TTA TTG TCG TTG GTT GTA CGA GAA
Met Asp Leu Asp Glu Ala Leu Gln Asn Asn Ser Asn Gln His Ala Leu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      440      450      460      470      480
      *      *      *      *      *      *      *      *
GCT AAA GCT GGC TTG GAG CTA ACA AAT TCA TTA ATT GAA AAT ATT GCT
CGA TTT CGA CCG AAC CTC GAT TGT TTA AGT AAT TAA CTT TTA TAA CGA
Ala Lys Ala Gly Leu Glu Leu Thr Asn Ser Leu Ile Glu Asn Ile Ala>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      490      500      510      520
      *      *      *      *      *      *      *      *
AAT TCA GTA AAA ACA CTT GAC GAA TTT GGT GAG CAA ATT AGT CAA TTT
TTA AGT CAT TTT TGT GAA CTG CTT AAA CCA CTC GTT TAA TCA GTT AAA
Asn Ser Val Lys Thr Leu Asp Glu Phe Gly Glu Gln Ile Ser Gln Phe>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

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FIG. 6B

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530          540          550          560          570
*           *           *           *           *
GGT TCA AAA CTA CAA AAT ATC AAA GGC TTA GGG ACT TTA GGA GAC AAA
CCA AGT TTT GAT GTT TTA TAG TTT CCG AAT CCC TGA AAT CCT CTG TTT
Gly Ser Lys Leu Gln Asn Ile Lys Gly Leu Gly Thr Leu Gly Asp Lys>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

580          590          600          610          620
*           *           *           *           *
CTC AAA AAT ATC GGT GGA CTT GAT AAA GCT GGC CTT GGT TTA GAT GTT
GAG TTT TTA TAG CCA CCT GAA CTA TTT CGA CCG GAA CCA AAT CTA CAA
Leu Lys Asn Ile Gly Gly Leu Asp Lys Ala Gly Leu Gly Leu Asp Val>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

630          640          650          660          670
*           *           *           *           *
ATC TCA GGG CTA TTA TCG GGC GCA ACA GCT GCA CTT GTA CTT GCA GAT
TAG AGT CCC GAT AAT AGC CCG CGT TGT CGA CGT GAA CAT GAA CGT CTA
Ile Ser Gly Leu Leu Ser Gly Ala Thr Ala Ala Leu Val Leu Ala Asp>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

680          690          700          710          720
*           *           *           *           *
AAA AAT GCT TCA ACA GCT AAA AAA GTG GGT GCG GGT TTT GAA TTG GCA
TTT TTA CGA AGT TGT CGA TTT TTT CAC CCA CGC CCA AAA CTT AAC CGT
Lys Asn Ala Ser Thr Ala Lys Lys Val Gly Ala Gly Phe Glu Leu Ala>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

730          740          750          760
*           *           *           *           *
AAC CAA GTT GTT GGT AAT ATT ACC AAA GCC GTT TCT TCT TAC ATT TTA
TTG GTT CAA CAA CCA TTA TAA TGG TTT CGG CAA AGA AGA ATG TAA AAT
Asn Gln Val Val Gly Asn Ile Thr Lys Ala Val Ser Ser Tyr Ile Leu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

770          780          790          800          810
*           *           *           *           *
GCC CAA CGT GTT GCA GCA GGT TTA TCT TCA ACT GGG CCT GTG GCT GCT
CGG GTT GCA CAA CGT CGT CCA AAT AGA AGT TGA CCC GGA CAC CGA CGA
Ala Gln Arg Val Ala Ala Gly Leu Ser Ser Thr Gly Pro Val Ala Ala>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

820          830          840          850          860
*           *           *           *           *
TTA ATT GCT TCT ACT GTT TCT CTT GCG ATT AGC CCA TTA GCA TTT GCC
AAT TAA CGA AGA TGA CAA AGA GAA CGC TAA TCG GGT AAT CGT AAA CGG
Leu Ile Ala Ser Thr Val Ser Leu Ala Ile Ser Pro Leu Ala Phe Ala>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

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FIG. 6C

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      870          880          890          900          910
*      *          *      *          *      *          *      *
GGT ATT GCC GAT AAA TTT AAT CAT GCA AAA AGT TTA GAG AGT TAT GCC
CCA TAA CGG CTA TTT AAA TTA GTA CGT TTT TCA AAT CTC TCA ATA CGG
Gly Ile Ala Asp Lys Phe Asn His Ala Lys Ser Leu Glu Ser Tyr Ala>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      920          930          940          950          960
*      *          *      *          *      *          *      *
GAA CGC TTT AAA AAA TTA GGC TAT GAC GGA GAT AAT TTA TTA GCA GAA
CTT GCG AAA TTT TTT AAT CCG ATA CTG CCT CTA TTA AAT AAT CGT CTT
Glu Arg Phe Lys Lys Leu Gly Tyr Asp Gly Asp Asn Leu Leu Ala Glu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      970          980          990          1000
*      *          *      *          *      *          *      *
TAT CAG CGG GGA ACA GGG ACT ATT GAT GCA TCG GTT ACT GCA ATT AAT
ATA GTC GCC CCT TGT CCC TGA TAA CTA CGT AGC CAA TGA CGT TAA TTA
Tyr Gln Arg Gly Thr Gly Thr Ile Asp Ala Ser Val Thr Ala Ile Asn>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1010          1020          1030          1040          1050
*      *          *      *          *      *          *      *
ACC GCA TTG GCC GCT ATT GCT GGT GGT GTG TCT GCT GCT GCA GCC GGC
TGG CGT AAC CGG CGA TAA CGA CCA CCA CAC AGA CGA CGA CGT CGG CCG
Thr Ala Leu Ala Ala Ile Ala Gly Gly Val Ser Ala Ala Ala Ala Gly>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1060          1070          1080          1090          1100
*      *          *      *          *      *          *      *
TCG GTT ATT GCT TCA CCG ATT GCC TTA TTA GTA TCT GGG ATT ACC GGT
AGC CAA TAA CGA AGT GGC TAA CGG AAT AAT CAT AGA CCC TAA TGG CCA
Ser Val Ile Ala Ser Pro Ile Ala Leu Leu Val Ser Gly Ile Thr Gly>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1110          1120          1130          1140          1150
*      *          *      *          *      *          *      *
GTA ATT TCT ACG ATT CTG CAA TAT TCT AAA CAA GCA ATG TTT GAG CAC
CAT TAA AGA TGC TAA GAC GTT ATA AGA TTT GTT CGT TAC AAA CTC GTG
Val Ile Ser Thr Ile Leu Gln Tyr Ser Lys Gln Ala Met Phe Glu His>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

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FIG. 6D

1160 1170 1180 1190 1200
 * * * * *
 GTT GCA AAT AAA ATT CAT AAC AAA ATT GTA GAA TGG GAA AAA AAT AAT
 CAA CGT TTA TTT TAA GTA TTG TTT TAA CAT CTT ACC CTT TTT TTA TTA
 Val Ala Asn Lys Ile His Asn Lys Ile Val Glu Trp Glu Lys Asn Asn>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1210 1220 1230 1240
 * * * * *
 CAC GGT AAG AAC TAC TTT GAA AAT GGT TAC GAT GCC CGT TAT CTT GCG
 GTG CCA TTC TTG ATG AAA CTT TTA CCA ATG CTA CGG GCA ATA GAA CGC
 His Gly Lys Asn Tyr Phe Glu Asn Gly Tyr Asp Ala Arg Tyr Leu Ala>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1250 1260 1270 1280 1290
 * * * * *
 AAT TTA CAA GAT AAT ATG AAA TTC TTA CTG AAC TTA AAC AAA GAG TTA
 TTA AAT GTT CTA TTA TAC TTT AAG AAT GAC TTG AAT TTG TTT CTC AAT
 Asn Leu Gln Asp Asn Met Lys Phe Leu Leu Asn Leu Asn Lys Glu Leu>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1300 1310 1320 1330 1340
 * * * * *
 CAG GCA GAA CGT GTC ATC GCT ATT ACT CAG CAG CAA TGG GAT AAC AAC
 GTC CGT CTT GCA CAG TAG CGA TAA TGA GTC GTC GTT ACC CTA TTG TTG
 Gln Ala Glu Arg Val Ile Ala Ile Thr Gln Gln Gln Trp Asp Asn Asn>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1350 1360 1370 1380 1390
 * * * * *
 ATT GGT GAT TTA GCT GGT ATT AGC CGT TTA GGT GAA AAA GTC CTT AGT
 TAA CCA CTA AAT CGA CCA TAA TCG GCA AAT CCA CTT TTT CAG GAA TCA
 Ile Gly Asp Leu Ala Gly Ile Ser Arg Leu Gly Glu Lys Val Leu Ser>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1400 1410 1420 1430 1440
 * * * * *
 GGT AAA GCC TAT GTG GAT GCG TTT GAA GAA GGC AAA CAC ATT AAA GCC
 CCA TTT CGG ATA CAC CTA CGC AAA CTT CTT CCG TTT GTG TAA TTT CGG
 Gly Lys Ala Tyr Val Asp Ala Phe Glu Glu Gly Lys His Ile Lys Ala>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1450 1460 1470 1480
 * * * * *
 GAT AAA TTA GTA CAG TTG GAT TCG GCA AAC GGT ATT ATT GAT GTG AGT
 CTA TTT AAT CAT GTC AAC CTA AGC CGT TTG CCA TAA TAA CTA CAC TCA
 Asp Lys Leu Val Gln Leu Asp Ser Ala Asn Gly Ile Ile Asp Val Ser>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

FIG. 6E

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1490          1500          1510          1520          1530
  *          *          *          *          *
AAT TCG GGT AAA GCG AAA ACT CAG CAT ATC TTA TTC AGA ACG CCA TTA
TTA AGC CCA TTT CGC TTT TGA GTC GTA TAG AAT AAG TCT TGC GGT AAT
Asn Ser Gly Lys Ala Lys Thr Gln His Ile Leu Phe Arg Thr Pro Leu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1540          1550          1560          1570          1580
  *          *          *          *          *
TTG ACG CCG GGA ACA GAG CAT CGT GAA CGC GTA CAA ACA GGT AAA TAT
AAC TGC GGC CCT TGT CTC GTA GCA CTT GCG CAT GTT TGT CCA TTT ATA
Leu Thr Pro Gly Thr Glu His Arg Glu Arg Val Gln Thr Gly Lys Tyr>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1590          1600          1610          1620          1630
  *          *          *          *          *
GAA TAT ATT ACC AAG CTC AAT ATT AAC CGT GTA GAT AGC TGG AAA ATT
CTT ATA TAA TGG TTC GAG TTA TAA TTG GCA CAT CTA TCG ACC TTT TAA
Glu Tyr Ile Thr Lys Leu Asn Ile Asn Arg Val Asp Ser Trp Lys Ile>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1640          1650          1660          1670          1680
  *          *          *          *          *
ACA GAT GGT GCA GCA AGT TCT ACC TTT GAT TTA ACT AAC GTT GTT CAG
TGT CTA CCA CGT CGT TCA AGA TGG AAA CTA AAT TGA TTG CAA CAA GTC
Thr Asp Gly Ala Ala Ser Ser Thr Phe Asp Leu Thr Asn Val Val Gln>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1690          1700          1710          1720
  *          *          *          *          *
CGT ATT GGT ATT GAA TTA GAC AAT GCT GGA AAT GTA ACT AAA ACC AAA
GCA TAA CCA TAA CTT AAT CTG TTA CGA CCT TTA CAT TGA TTT TGG TTT
Arg Ile Gly Ile Glu Leu Asp Asn Ala Gly Asn Val Thr Lys Thr Lys>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

1730          1740          1750          1760          1770
  *          *          *          *          *
GAA ACA AAA ATT ATT GCC AAA CTT GGT GAA GGT GAT GAC AAC GTA TTT
CTT TGT TTT TAA TAA CGG TTT GAA CCA CTT CCA CTA CTG TTG CAT AAA
Glu Thr Lys Ile Ile Ala Lys Leu Gly Glu Gly Asp Asp Asn Val Phe>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

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FIG. 6F

1780 1790 1800 1810 1820
 * * * * * *
 GTT GGT TCT GGT ACG ACG GAA ATT GAT GGC GGT GAA GGT TAC GAC CGA
 CAA CCA AGA CCA TGC TGC CTT TAA CTA CCG CCA CTT CCA ATG CTG GCT
 Val Gly Ser Gly Thr Thr Glu Ile Asp Gly Gly Glu Gly Tyr Asp Arg>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1830 1840 1850 1860 1870
 * * * * * *
 GTT CAC TAT AGC CGT GGA AAC TAT GGT GCT TTA ACT ATT GAT GCA ACC
 CAA GTG ATA TCG GCA CCT TTG ATA CCA CGA AAT TGA TAA CTA CGT TGG
 Val His Tyr Ser Arg Gly Asn Tyr Gly Ala Leu Thr Ile Asp Ala Thr>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1880 1890 1900 1910 1920
 * * * * * *
 AAA GAG ACC GAG CAA GGT AGT TAT ACC GTA AAT CGT TTC GTA GAA ACC
 TTT CTC TGG CTC GTT CCA TCA ATA TGG CAT TTA GCA AAG CAT CTT TGG
 Lys Glu Thr Glu Gln Gly Ser Tyr Thr Val Asn Arg Phe Val Glu Thr>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1930 1940 1950 1960
 * * * * * *
 GGT AAA GCA CTA CAC GAA GTG ACT TCA ACC CAT ACC GCA TTA GTG GGC
 CCA TTT CGT GAT GTG CTT CAC TGA AGT TGG GTA TGG CGT AAT CAC CCG
 Gly Lys Ala Leu His Glu Val Thr Ser Thr His Thr Ala Leu Val Gly>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

1970 1980 1990 2000 2010
 * * * * * *
 AAC CGT GAA GAA AAA ATA GAA TAT CGT CAT AGC AAT AAC CAG CAC CAT
 TTG GCA CTT CTT TTT TAT CTT ATA GCA GTA TCG TTA TTG GTC GTG GTA
 Asn Arg Glu Glu Lys Ile Glu Tyr Arg His Ser Asn Asn Gln His His>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2020 2030 2040 2050 2060
 * * * * * *
 GCC GGT TAT TAC ACC AAA GAT ACC TTG AAA GCT GTT GAA GAA ATT ATC
 CGG CCA ATA ATG TGG TTT CTA TGG AAC TTT CGA CAA CTT CTT TAA TAG
 Ala Gly Tyr Tyr Thr Lys Asp Thr Leu Lys Ala Val Glu Glu Ile Ile>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

2070 2080 2090 2100 2110
 * * * * * *
 GGT ACA TCA CAT AAC GAT ATC TTT AAA GGT AGT AAG TTC AAT GAT GCC
 CCA TGT AGT GTA TTG CTA TAG AAA TTT CCA TCA TTC AAG TTA CTA CGG
 Gly Thr Ser His Asn Asp Ile Phe Lys Gly Ser Lys Phe Asn Asp Ala>
 ___a___a___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a___a___a___>

FIG. 6G

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      2120      2130      2140      2150      2160
      *      *      *      *      *      *      *      *
TTT AAC GGT GGT GAT GGT GTC GAT ACT ATT GAC GGT AAC GAC GGC AAT
AAA TTG CCA CCA CTA CCA CAG CTA TGA TAA CTG CCA TTG CTG CCG TTA
Phe Asn Gly Gly Asp Gly Val Asp Thr Ile Asp Gly Asn Asp Gly Asn>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      2170      2180      2190      2200
      *      *      *      *      *      *      *      *
GAC CGC TTA TTT GGT GGT AAA GGC GAT GAT ATT CTC GAT GGT GGA AAT
CTG GCG AAT AAA CCA CCA TTT CCG CTA CTA TAA GAG CTA CCA CCT TTA
Asp Arg Leu Phe Gly Gly Lys Gly Asp Asp Ile Leu Asp Gly Gly Asn>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

2210      2220      2230      2240      2250
      *      *      *      *      *      *      *      *
GGT GAT GAT TTT ATC GAT GGC GGT AAA GGC AAC GAC CTA TTA CAC GGT
CCA CTA CTA AAA TAG CTA CCG CCA TTT CCG TTG CTG GAT AAT GTG CCA
Gly Asp Asp Phe Ile Asp Gly Gly Lys Gly Asn Asp Leu Leu His Gly>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      2260      2270      2280      2290      2300
      *      *      *      *      *      *      *      *
GGC AAG GGC GAT GAT ATT TTC GTT CAC CGT AAA GGC GAT GGT AAT GAT
CCG TTC CCG CTA CTA TAA AAG CAA GTG GCA TTT CCG CTA CCA TTA CTA
Gly Lys Gly Asp Asp Ile Phe Val His Arg Lys Gly Asp Gly Asn Asp>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      2310      2320      2330      2340      2350
      *      *      *      *      *      *      *      *
ATT ATT ACC GAT TCT GAC GGC AAT GAT AAA TTA TCA TTC TCT GAT TCG
TAA TAA TGG CTA AGA CTG CCG TTA CTA TTT AAT AGT AAG AGA CTA AGC
Ile Ile Thr Asp Ser Asp Gly Asn Asp Lys Leu Ser Phe Ser Asp Ser>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      2360      2370      2380      2390      2400
      *      *      *      *      *      *      *      *
AAC TTA AAA GAT TTA ACA TTT GAA AAA GTT AAA CAT AAT CTT GTC ATC
TTG AAT TTT CTA AAT TGT AAA CTT TTT CAA TTT GTA TTA GAA CAG TAG
Asn Leu Lys Asp Leu Thr Phe Glu Lys Val Lys His Asn Leu Val Ile>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

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FIG. 6H

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      2410      2420      2430      2440
      *      *      *      *      *      *      *      *
ACG AAT AGC AAA AAA GAG AAA GTG ACC ATT CAA AAC TGG TTC CGA GAG
TGC TTA TCG TTT TTT CTC TTT CAC TGG TAA GTT TTG ACC AAG GCT CTC
Thr Asn Ser Lys Lys Glu Lys Val Thr Ile Gln Asn Trp Phe Arg Glu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

2450      2460      2470      2480      2490
      *      *      *      *      *      *      *      *
GCT GAT TTT GCT AAA GAA GTG CCT AAT TAT AAA GCA ACT AAA GAT GAG
CGA CTA AAA CGA TTT CTT CAC GGA TTA ATA TTT CGT TGA TTT CTA CTC
Ala Asp Phe Ala Lys Glu Val Pro Asn Tyr Lys Ala Thr Lys Asp Glu>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      2500      2510      2520      2530      2540
      *      *      *      *      *      *      *      *
AAA ATC GAA GAA ATC ATC GGT CAA AAT GGC GAG CGG ATC ACC TCA AAG
TTT TAG CTT CTT TAG TAG CCA GTT TTA CCG CTC GCC TAG TGG AGT TTC
Lys Ile Glu Glu Ile Ile Gly Gln Asn Gly Glu Arg Ile Thr Ser Lys>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      2550      2560      2570      2580      2590
      *      *      *      *      *      *      *      *
CAA GTT GAT GAT CTT ATC GCA AAA GGT AAC GGC AAA ATT ACC CAA GAT
GTT CAA CTA CTA GAA TAG CGT TTT CCA TTG CCG TTT TAA TGG GTT CTA
Gln Val Asp Asp Leu Ile Ala Lys Gly Asn Gly Lys Ile Thr Gln Asp>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      2600      2610      2620      2630      2640
      *      *      *      *      *      *      *      *
GAG CTA TCA AAA GTT GTT GAT AAC TAT GAA TTG CTC AAA CAT AGC AAA
CTC GAT AGT TTT CAA CAA CTA TTG ATA CTT AAC GAG TTT GTA TCG TTT
Glu Leu Ser Lys Val Val Asp Asn Tyr Glu Leu Leu Lys His Ser Lys>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

      2650      2660      2670      2680
      *      *      *      *      *      *      *      *
AAT GTG ACA AAC AGC TTA GAT AAG TTA ATC TCA TCT GTA AGT GCA TTT
TTA CAC TGT TTG TCG AAT CTA TTC AAT TAG AGT AGA CAT TCA CGT AAA
Asn Val Thr Asn Ser Leu Asp Lys Leu Ile Ser Ser Val Ser Ala Phe>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

2690      2700      2710      2720      2730
      *      *      *      *      *      *      *      *
ACC TCG TCT AAT GAT TCG AGA AAT GTA TTA GTG GCT CCA ACT TCA ATG
TGG AGC AGA TTA CTA AGC TCT TTA CAT AAT CAC CGA GGT TGA AGT TAC
Thr Ser Ser Asn Asp Ser Arg Asn Val Leu Val Ala Pro Thr Ser Met>
__a__a__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__a__>

```

FIG. 6I

CC44217" 99592680

```
2740      2750      2760      2770      2780
  *      *      *      *      *
TTG GAT CAA AGT TTA TCT TCT CTT CAA TTT GCT AGG GGA TCC AGC TCT
AAC CTA GTT TCA AAT AGA AGA GAA GTT AAA CGA TCC CCT AGG TCG AGA
Leu Asp Gln Ser Leu Ser Ser Leu Gln Phe Ala Arg Gly Ser>
__a__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_a__a__>
                                           Ser Ser>
                                           __b__>

2790      2800      2810      2820      2830
  *      *      *      *      *
TCT GCC GGC TGC AAA AAC TTC TTC TGG AAA ACC TTC ACC AGC TGC TAG
AGA CGG CCG ACG TTT TTG AAG AAG ACC TTT TGG AAG TGG TCG ACG ATC
Ser>
__>
Ala Gly Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys End>
__c__c__c__c__c__SRIF PEPTIDE__c__c__c__c__c__>
```

*
GGATCC
CCTAGG

FIG. 6J

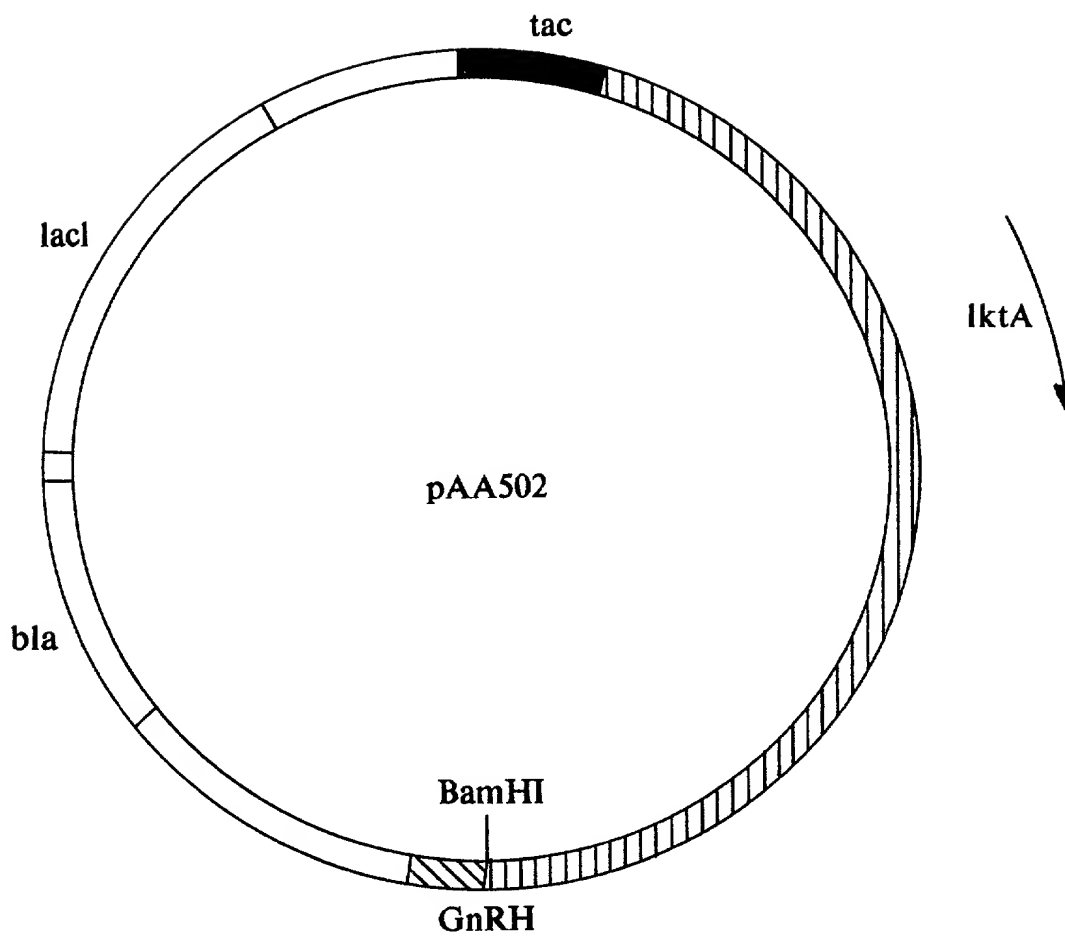


FIG. 7

08976566 "112497"

```

      10      20      30      40
      *      *      *      *      *      *      *      *
ATG GCT ACT GTT ATA GAT CTA AGC TTC CCA AAA ACT GGG GCA AAA AAA
TAC CGA TGA CAA TAT CTA GAT TCG AAG GGT TTT TGA CCC CGT TTT TTT
Met Ala Thr Val Ile Asp Leu Ser Phe Pro Lys Thr Gly Ala Lys Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

50      60      70      80      90
      *      *      *      *      *      *      *      *
ATT ATC CTC TAT ATT CCC CAA AAT TAC CAA TAT GAT ACT GAA CAA GGT
TAA TAG GAG ATA TAA GGG GTT TTA ATG GTT ATA CTA TGA CTT GTT CCA
Ile Ile Leu Tyr Ile Pro Gln Asn Tyr Gln Tyr Asp Thr Glu Gln Gly>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

100     110     120     130     140
      *      *      *      *      *      *      *      *
AAT GGT TTA CAG GAT TTA GTC AAA GCG GCC GAA GAG TTG GGG ATT GAG
TTA CCA AAT GTC CTA AAT CAG TTT CGC CGG CTT CTC AAC CCC TAA CTC
Asn Gly Leu Gln Asp Leu Val Lys Ala Ala Glu Glu Leu Gly Ile Glu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

150     160     170     180     190
      *      *      *      *      *      *      *      *
GTA CAA AGA GAA GAA CGC AAT AAT ATT GCA ACA GCT CAA ACC AGT TTA
CAT GTT TCT CTT CTT GCG TTA TTA TAA CGT TGT CGA GTT TGG TCA AAT
Val Gln Arg Glu Glu Arg Asn Asn Ile Ala Thr Ala Gln Thr Ser Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

200     210     220     230     240
      *      *      *      *      *      *      *      *
GGC ACG ATT CAA ACC GCT ATT GGC TTA ACT GAG CGT GGC ATT GTG TTA
CCG TGC TAA GTT TGG CGA TAA CCG AAT TGA CTC GCA CCG TAA CAC AAT
Gly Thr Ile Gln Thr Ala Ile Gly Leu Thr Glu Arg Gly Ile Val Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

FIG. 8A

```

                250                260                270                280
      *      *      *      *      *      *      *      *      *
TCC GCT CCA CAA ATT GAT AAA TTG CTA CAG AAA ACT AAA GCA GGC CAA
AGG CGA GGT GTT TAA CTA TTT AAC GAT GTC TTT TGA TTT CGT CCG GTT
Ser Ala Pro Gln Ile Asp Lys Leu Leu Gln Lys Thr Lys Ala Gly Gln>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

290                300                310                320                330
      *      *      *      *      *      *      *      *      *
GCA TTA GGT TCT GCC GAA AGC ATT GTA CAA AAT GCA AAT AAA GCC AAA
CGT AAT CCA AGA CCG CTT TCG TAA CAT GTT TTA CGT TTA TTT CGG TTT
Ala Leu Gly Ser Ala Glu Ser Ile Val Gln Asn Ala Asn Lys Ala Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

340                350                360                370                380
      *      *      *      *      *      *      *      *      *
ACT GTA TTA TCT GGC ATT CAA TCT ATT TTA GGC TCA GTA TTG GCT GGA
TGA CAT AAT AGA CCG TAA GTT AGA TAA AAT CCG AGT CAT AAC CGA CCT
Thr Val Leu Ser Gly Ile Gln Ser Ile Leu Gly Ser Val Leu Ala Gly>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

390                400                410                420                430
      *      *      *      *      *      *      *      *      *
ATG GAT TTA GAT GAG GCC TTA CAG AAT AAC AGC AAC CAA CAT GCT CTT
TAC CTA AAT CTA CTC CGG AAT GTC TTA TTG TCG TTG GTT GTA CGA GAA
Met Asp Leu Asp Glu Ala Leu Gln Asn Asn Ser Asn Gln His Ala Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

440                450                460                470                480
      *      *      *      *      *      *      *      *      *
GCT AAA GCT GGC TTG GAG CTA ACA AAT TCA TTA ATT GAA AAT ATT GCT
CGA TTT CGA CCG AAC CTC GAT TGT TTA AGT AAT TAA CTT TTA TAA CGA
Ala Lys Ala Gly Leu Glu Leu Thr Asn Ser Leu Ile Glu Asn Ile Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

490                500                510                520
      *      *      *      *      *      *      *      *      *
AAT TCA GTA AAA ACA CTT GAC GAA TTN -GT GAG CAA ATT AGT CAA TTT
TTA AGT CAT TTT TGT GAA CTG CTT AAN -CA CTC GTT TAA TCA GTT AAA
Asn Ser Val Lys Thr Leu Asp Glu Xxx Cys Glu Gln Ile Ser Gln Phe>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

530                540                550                560                570
      *      *      *      *      *      *      *      *      *
GGT TCA AAA CTA CAA AAT ATC AAA GGC TTA GGG ACT TTA GGA GAC AAA
CCA AGT TTT GAT GTT TTA TAG TTT CCG AAT CCC TGA AAT CCT CTG TTT
Gly Ser Lys Leu Gln Asn Ile Lys Gly Leu Gly Thr Leu Gly Asp Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

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FIG. 8B

```

      580          590          600          610          620
      *          *          *          *          *
CTC AAA AAT ATC GGT GGA CTT GAT AAA GCT GGC CTT GGT TTA GAT GTT
GAG TTT TTA TAG CCA CCT GAA CTA TTT CGA CCG GAA CCA AAT CTA CAA
Leu Lys Asn Ile Gly Gly Leu Asp Lys Ala Gly Leu Gly Leu Asp Val>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      630          640          650          660          670
      *          *          *          *          *
ATC TCA GGG CTA TTA TCG GGC GCA ACA GCT GCA CTT GTA CTT GCA GAT
TAG AGT CCC GAT AAT AGC CCG CGT TGT CGA CGT GAA CAT GAA CGT CTA
Ile Ser Gly Leu Leu Ser Gly Ala Thr Ala Ala Leu Val Leu Ala Asp>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      680          690          700          710          720
      *          *          *          *          *
AAA AAT GCT TCA ACA GCT AAA AAA GTG GGT GCG GGT TTT GAA TTG GCA
TTT TTA CGA AGT TGT CGA TTT TTT CAC CCA CGC CCA AAA CTT AAC CGT
Lys Asn Ala Ser Thr Ala Lys Lys Val Gly Ala Gly Phe Glu Leu Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      730          740          750          760
      *          *          *          *          *
AAC CAA GTT GTT GGT AAT ATT ACC AAA GCC GTT TCT TCT TAC ATT TTA
TTG GTT CAA CAA CCA TTA TAA TGG TTT CGG CAA AGA AGA ATG TAA AAT
Asn Gln Val Val Gly Asn Ile Thr Lys Ala Val Ser Ser Tyr Ile Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      770          780          790          800          810
      *          *          *          *          *
GCC CAA CGT GTT GCA GCA GGT TTA TCT TCA ACT GGG CCT GTG GCT GCT
CGG GTT GCA CAA CGT CGT CCA AAT AGA AGT TGA CCC GGA CAC CGA CGA
Ala Gln Arg Val Ala Ala Gly Leu Ser Ser Thr Gly Pro Val Ala Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      820          830          840          850          860
      *          *          *          *          *
TTA ATT GCT TCT ACT GTT TCT CTT GCG ATT AGC CCA TTA GCA TTT GCC
AAT TAA CGA AGA TGA CAA AGA GAA CGC TAA TCG GGT AAT CGT AAA CGG
Leu Ile Ala Ser Thr Val Ser Leu Ala Ile Ser Pro Leu Ala Phe Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

FIG. 8C

```

      870      880      890      900      910
*      *      *      *      *      *      *
GGT ATT GCC GAT AAA TTT AAT CAT GCA AAA AGT TTA GAG AGT TAT GCC
CCA TAA CGG CTA TTT AAA TTA GTA CGT TTT TCA AAT CTC TCA ATA CGG
Gly Ile Ala Asp Lys Phe Asn His Ala Lys Ser Leu Glu Ser Tyr Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      920      930      940      950      960
*      *      *      *      *      *      *
GAA CGC TTT AAA AAA TTA GGC TAT GAC GGA GAT AAT TTA TTA GCA GAA
CTT GCG AAA TTT TTT AAT CCG ATA CTG CCT CTA TTA AAT AAT CGT CTT
Glu Arg Phe Lys Lys Leu Gly Tyr Asp Gly Asp Asn Leu Leu Ala Glu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      970      980      990      1000
*      *      *      *      *      *      *
TAT CAG CGG GGA ACA GGG ACT ATT GAT GCA TCG GTT ACT GCA ATT AAT
ATA GTC GCC CCT TGT CCC TGA TAA CTA CGT AGC CAA TGA CGT TAA TTA
Tyr Gln Arg Gly Thr Gly Thr Ile Asp Ala Ser Val Thr Ala Ile Asn>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1010      1020      1030      1040      1050
*      *      *      *      *      *      *
ACC GCA TTG GCC GCT ATT GCT GGT GGT GTG TCT GCT GCT GCA GCC GGC
TGG CGT AAC CGG CGA TAA CGA CCA CCA CAC AGA CGA CGA CGT CGG CCG
Thr Ala Leu Ala Ala Ile Ala Gly Gly Val Ser Ala Ala Ala Ala Gly>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1060      1070      1080      1090      1100
*      *      *      *      *      *      *
TCG GTT ATT GCT TCA CCG ATT GCC TTA TTA GTA TCT GGG ATT ACC GGT
AGC CAA TAA CGA AGT GGC TAA CGG AAT AAT CAT AGA CCC TAA TGG CCA
Ser Val Ile Ala Ser Pro Ile Ala Leu Leu Val Ser Gly Ile Thr Gly>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1110      1120      1130      1140      1150
*      *      *      *      *      *      *
GTA ATT TCT ACG ATT CTG CAA TAT TCT AAA CAA GCA ATG TTT GAG CAC
CAT TAA AGA TGC TAA GAC GTT ATA AGA TTT GTT CGT TAC AAA CTC GTG
Val Ile Ser Thr Ile Leu Gln Tyr Ser Lys Gln Ala Met Phe Glu His>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1160      1170      1180      1190      1200
*      *      *      *      *      *      *
GTT GCA AAT AAA ATT CAT AAC AAA ATT GTA GAA TGG GAA AAA AAT AAT
CAA CGT TTA TTT TAA GTA TTG TTT TAA CAT CTT ACC CTT TTT TTA TTA
Val Ala Asn Lys Ile His Asn Lys Ile Val Glu Trp Glu Lys Asn Asn>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

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FIG. 8D

08976566-11249

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      1210      1220      1230      1240
      *      *      *      *
CAC GGT AAG AAC TAC TTT GAA AAT GGT TAC GAT GCC CGT TAT CTT GCG
GTG CCA TTC TTG ATG AAA CTT TTA CCA ATG CTA CGG GCA ATA GAA CGC
His Gly Lys Asn Tyr Phe Glu Asn Gly Tyr Asp Ala Arg Tyr Leu Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]__b__b__b__>

1250      1260      1270      1280      1290
      *      *      *      *      *
AAT TTA CAA GAT AAT ATG AAA TTC TTA CTG AAC TTA AAC AAA GAG TTA
TTA AAT GTT CTA TTA TAC TTT AAG AAT GAC TTG AAT TTG TTT CTC AAT
Asn Leu Gln Asp Asn Met Lys Phe Leu Leu Asn Leu Asn Lys Glu Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]__b__b__b__>

      1300      1310      1320      1330      1340
      *      *      *      *      *
CAG GCA GAA CGT GTC ATC GCT ATT ACT CAG CAG CAA TGG GAT AAC AAC
GTC CGT CTT GCA CAG TAG CGA TAA TGA GTC GTC GTT ACC CTA TTG TTG
Gln Ala Glu Arg Val Ile Ala Ile Thr Gln Gln Gln Trp Asp Asn Asn>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]__b__b__b__>

      1350      1360      1370      1380      1390
      *      *      *      *      *
ATT GGT GAT TTA GCT GGT ATT AGC CGT TTA GGT GAA AAA GTC CTT AGT
TAA CCA CTA AAT CGA CCA TAA TCG GCA AAT CCA CTT TTT CAG GAA TCA
Ile Gly Asp Leu Ala Gly Ile Ser Arg Leu Gly Glu Lys Val Leu Ser>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]__b__b__b__>

      1400      1410      1420      1430      1440
      *      *      *      *      *
GGT AAA GCC TAT GTG GAT GCG TTT GAA GAA GGC AAA CAC ATT AAA GCC
CCA TTT CGG ATA CAC CTA CGC AAA CTT CTT CCG TTT GTG TAA TTT CGG
Gly Lys Ala Tyr Val Asp Ala Phe Glu Glu Gly Lys His Ile Lys Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]__b__b__b__>

      1450      1460      1470      1480
      *      *      *      *      *
GAT AAA TTA GTA CAG TTG GAT TCG GCA AAC GGT ATT ATT GAT GTG AGT
CTA TTT AAT CAT GTC AAC CTA AGC CGT TTG CCA TAA TAA CTA CAC TCA
Asp Lys Leu Val Gln Leu Asp Ser Ala Asn Gly Ile Ile Asp Val Ser>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]__b__b__b__>

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FIG. 8E

1490 1500 1510 1520 1530
 * * * * * * *
 AAT TCG GGT AAA GCG AAA ACT CAG CAT ATC TTA TTC AGA ACG CCA TTA
 TTA AGC CCA TTT CGC TTT TGA GTC GTA TAG AAT AAG TCT TGC GGT AAT
 Asn Ser Gly Lys Ala Lys Thr Gln His Ile Leu Phe Arg Thr Pro Leu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1540 1550 1560 1570 1580
 * * * * * * *
 TTG ACG CCG GGA ACA GAG CAT CGT GAA CGC GTA CAA ACA GGT AAA TAT
 AAC TGC GGC CCT TGT CTC GTA GCA CTT GCG CAT GTT TGT CCA TTT ATA
 Leu Thr Pro Gly Thr Glu His Arg Glu Arg Val Gln Thr Gly Lys Tyr>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1590 1600 1610 1620 1630
 * * * * * * *
 GAA TAT ATT ACC AAG CTC AAT ATT AAC CGT GTA GAT AGC TGG AAA ATT
 CTT ATA TAA TGG TTC GAG TTA TAA TTG GCA CAT CTA TCG ACC TTT TAA
 Glu Tyr Ile Thr Lys Leu Asn Ile Asn Arg Val Asp Ser Trp Lys Ile>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1640 1650 1660 1670 1680
 * * * * * * *
 ACA GAT GGT GCA GCA AGT TCT ACC TTT GAT TTA ACT AAC GTT GTT CAG
 TGT CTA CCA CGT CGT TCA AGA TGG AAA CTA AAT TGA TTG CAA CAA GTC
 Thr Asp Gly Ala Ala Ser Ser Thr Phe Asp Leu Thr Asn Val Val Gln>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1690 1700 1710 1720
 * * * * * * *
 CGT ATT GGT ATT GAA TTA GAC AAT GCT GGA AAT GTA ACT AAA ACC AAA
 GCA TAA CCA TAA CTT AAT CTG TTA CGA CCT TTA CAT TGA TTT TGG TTT
 Arg Ile Gly Ile Glu Leu Asp Asn Ala Gly Asn Val Thr Lys Thr Lys>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1730 1740 1750 1760 1770
 * * * * * * *
 GAA ACA AAA ATT ATT GCC AAA CTT GGT GAA GGT GAT GAC AAC GTA TTT
 CTT TGT TTT TAA TAA CGG TTT GAA CCA CTT CCA CTA CTG TTG CAT AAA
 Glu Thr Lys Ile Ile Ala Lys Leu Gly Glu Gly Asp Asp Asn Val Phe>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1780 1790 1800 1810 1820
 * * * * * * *
 GTT GGT TCT GGT ACG ACG GAA ATT GAT GGC GGT GAA GGT TAC GAC CGA
 CAA CCA AGA CCA TGC TGC CTT TAA CTA CCG CCA CTT CCA ATG CTG GCT
 Val Gly Ser Gly Thr Thr Glu Ile Asp Gly Gly Glu Gly Tyr Asp Arg>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

FIG. 8F

1830	1840	1850	1860	1870
* *	* *	* *	* *	* *
GTT CAC TAT AGC CGT GGA AAC TAT GGT GCT TTA ACT ATT GAT GCA ACC				
CAA GTG ATA TCG GCA CCT TTG ATA CCA CGA AAT TGA TAA CTA CGT TGG				
Val His Tyr Ser Arg Gly Asn Tyr Gly Ala Leu Thr Ile Asp Ala Thr>				
___b___b___	RECOMBINANT	LEUKOTOXIN PEPTIDE	[SPLIT]_b___b___b___>	

1880	1890	1900	1910	1920
* *	* *	* *	* *	* *
AAA GAG ACC GAG CAA GGT AGT TAT ACC GTA AAT CGT TTC GTA GAA ACC				
TTT CTC TGG CTC GTT CCA TCA ATA TGG CAT TTA GCA AAG CAT CTT TGG				
Lys Glu Thr Glu Gln Gly Ser Tyr Thr Val Asn Arg Phe Val Glu Thr>				
___b___b___	RECOMBINANT	LEUKOTOXIN PEPTIDE	[SPLIT]_b___b___b___>	

1930	1940	1950	1960
* *	* *	* *	* *
GGT AAA GCA CTA CAC GAA GTG ACT TCA ACC CAT ACC GCA TTA GTG GGC			
CCA TTT CGT GAT GTG CTT CAC TGA AGT TGG GTA TGG CGT AAT CAC CCG			
Gly Lys Ala Leu His Glu Val Thr Ser Thr His Thr Ala Leu Val Gly>			
___b___b___	RECOMBINANT	LEUKOTOXIN PEPTIDE	[SPLIT]_b___b___b___>

1970	1980	1990	2000	2010
* *	* *	* *	* *	* *
AAC CGT GAA GAA AAA ATA GAA TAT CGT CAT AGC AAT AAC CAG CAC CAT				
TTG GCA CTT CTT TTT TAT CTT ATA GCA GTA TCG TTA TTG GTC GTG GTA				
Asn Arg Glu Glu Lys Ile Glu Tyr Arg His Ser Asn Asn Gln His His>				
___b___b___	RECOMBINANT	LEUKOTOXIN PEPTIDE	[SPLIT]_b___b___b___>	

2020	2030	2040	2050	2060
* *	* *	* *	* *	* *
GCC GGT TAT TAC ACC AAA GAT ACC TTG AAA GCT GTT GAA GAA ATT ATC				
CGG CCA ATA ATG TGG TTT CTA TGG AAC TTT CGA CAA CTT CTT TAA TAG				
Ala Gly Tyr Tyr Thr Lys Asp Thr Leu Lys Ala Val Glu Glu Ile Ile>				
___b___b___	RECOMBINANT	LEUKOTOXIN PEPTIDE	[SPLIT]_b___b___b___>	

2070	2080	2090	2100	2110
* *	* *	* *	* *	* *
GGT ACA TCA CAT AAC GAT ATC TTT AAA GGT AGT AAG TTC AAT GAT GCC				
CCA TGT AGT GTA TTG CTA TAG AAA TTT CCA TCA TTC AAG TTA CTA CGG				
Gly Thr Ser His Asn Asp Ile Phe Lys Gly Ser Lys Phe Asn Asp Ala>				
___b___b___	RECOMBINANT	LEUKOTOXIN PEPTIDE	[SPLIT]_b___b___b___>	

FIG. 8G

```

      2120      2130      2140      2150      2160
      *      *      *      *      *      *      *      *
TTT AAC GGT GGT GAT GGT GTC GAT ACT ATT GAC GGT AAC GAC GGC AAT
AAA TTG CCA CCA CTA CCA CAG CTA TGA TAA CTG CCA TTG CTG CCG TTA
Phe Asn Gly Gly Asp Gly Val Asp Thr Ile Asp Gly Asn Asp Gly Asn>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2170      2180      2190      2200
      *      *      *      *      *      *      *      *
GAC CGC TTA TTT GGT GGT AAA GGC GAT GAT ATT CTC GAT GGT GGA AAT
CTG GCG AAT AAA CCA CCA TTT CCG CTA CTA TAA GAG CTA CCA CCT TTA
Asp Arg Leu Phe Gly Gly Lys Gly Asp Asp Ile Leu Asp Gly Gly Asn>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2210      2220      2230      2240      2250
      *      *      *      *      *      *      *      *
GGT GAT GAT TTT ATC GAT GGC GGT AAA GGC AAC GAC CTA TTA CAC GGT
CCA CTA CTA AAA TAG CTA CCG CCA TTT CCG TTG CTG GAT AAT GTG CCA
Gly Asp Asp Phe Ile Asp Gly Gly Lys Gly Asn Asp Leu Leu His Gly>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2260      2270      2280      2290      2300
      *      *      *      *      *      *      *      *
GGC AAG GGC GAT GAT ATT TTC GTT CAC CGT AAA GGC GAT GGT AAT GAT
CCG TTC CCG CTA CTA TAA AAG CAA GTG GCA TTT CCG CTA CCA TTA CTA
Gly Lys Gly Asp Asp Ile Phe Val His Arg Lys Gly Asp Gly Asn Asp>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2310      2320      2330      2340      2350
      *      *      *      *      *      *      *      *
ATT ATT ACC GAT TCT GAC GGC AAT GAT AAA TTA TCA TTC TCT GAT TCG
TAA TAA TGG CTA AGA CTG CCG TTA CTA TTT AAT AGT AAG AGA CTA AGC
Ile Ile Thr Asp Ser Asp Gly Asn Asp Lys Leu Ser Phe Ser Asp Ser>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2360      2370      2380      2390      2400
      *      *      *      *      *      *      *      *
AAC TTA AAA GAT TTA ACA TTT GAA AAA GTT AAA CAT AAT CTT GTC ATC
TTG AAT TTT CTA AAT TGT AAA CTT TTT CAA TTT GTA TTA GAA CAG TAG
Asn Leu Lys Asp Leu Thr Phe Glu Lys Val Lys His Asn Leu Val Ile>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2410      2420      2430      2440
      *      *      *      *      *      *      *      *
ACG AAT AGC AAA AAA GAG AAA GTG ACC ATT CAA AAC TGG TTC CGA GAG
TGC TTA TCG TTT TTT CTC TTT CAC TGG TAA GTT TTG ACC AAG GCT CTC
Thr Asn Ser Lys Lys Glu Lys Val Thr Ile Gln Asn Trp Phe Arg Glu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

FIG. 8H

```

2450          2460          2470          2480          2490
  *          *          *          *          *
GCT GAT TTT GCT AAA GAA GTG CCT AAT TAT AAA GCA ACT AAA GAT GAG
CGA CTA AAA CGA TTT CTT CAC GGA TTA ATA TTT CGT TGA TTT CTA CTC
Ala Asp Phe Ala Lys Glu Val Pro Asn Tyr Lys Ala Thr Lys Asp Glu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2500          2510          2520          2530          2540
  *          *          *          *          *
AAA ATC GAA GAA ATC ATC GGT CAA AAT GGC GAG CGG ATC ACC TCA AAG
TTT TAG CTT CTT TAG TAG CCA GTT TTA CCG CTC GCC TAG TGG AGT TTC
Lys Ile Glu Glu Ile Ile Gly Gln Asn Gly Glu Arg Ile Thr Ser Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2550          2560          2570          2580          2590
  *          *          *          *          *
CAA GTT GAT GAT CTT ATC GCA AAA GGT AAC GGC AAA ATT ACC CAA GAT
GTT CAA CTA CTA GAA TAG CGT TTT CCA TTG CCG TTT TAA TGG GTT CTA
Gln Val Asp Asp Leu Ile Ala Lys Gly Asn Gly Lys Ile Thr Gln Asp>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2600          2610          2620          2630          2640
  *          *          *          *          *
GAG CTA TCA AAA GTT GTT GAT AAC TAT GAA TTG CTC AAA CAT AGC AAA
CTC GAT AGT TTT CAA CAA CTA TTG ATA CTT AAC GAG TTT GTA TCG TTT
Glu Leu Ser Lys Val Val Asp Asn Tyr Glu Leu Leu Lys His Ser Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2650          2660          2670          2680
  *          *          *          *          *
AAT GTG ACA AAC AGC TTA GAT AAG TTA ATC TCA TCT GTA AGT GCA TTT
TTA CAC TGT TTG TCG AAT CTA TTC AAT TAG AGT AGA CAT TCA CGT AAA
Asn Val Thr Asn Ser Leu Asp Lys Leu Ile Ser Ser Val Ser Ala Phe>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2690          2700          2710          2720          2730
  *          *          *          *          *
ACC TCG TCT AAT GAT TCG AGA AAT GTA TTA GTG GCT CCA ACT TCA ATG
TGG AGC AGA TTA CTA AGC TCT TTA CAT AAT CAC CGA GGT TGA AGT TAC
Thr Ser Ser Asn Asp Ser Arg Asn Val Leu Val Ala Pro Thr Ser Met>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

FIG. 8I

```

      2740      2750      2760      2770      2780
      *      *      *      *      *
TTG GAT CAA AGT TTA TCT TCT CTT CAA TTT GCT AGG GGA TCT CAG CAT
AAC CTA GTT TCA AAT AGA AGA GAA GTT AAA CGA TCC CCT AGA GTC GTA
                                           Gln His>
Leu Asp Gln Ser Leu Ser Ser Leu Gln Phe Ala Arg Gly Ser>
__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__>

      2790      2800      2810
      *      *      *      *      *
TGG AGC TAC GGC CTG CGC CCT GGC TAA GGATCC
ACC TCG ATG CCG GAC GCG GGA CCG ATT CCTAGG
Trp Ser Tyr Gly Leu Arg Pro Gly End>
__a__a__a__GNRH__a__a__a__>

```

FIG. 8J

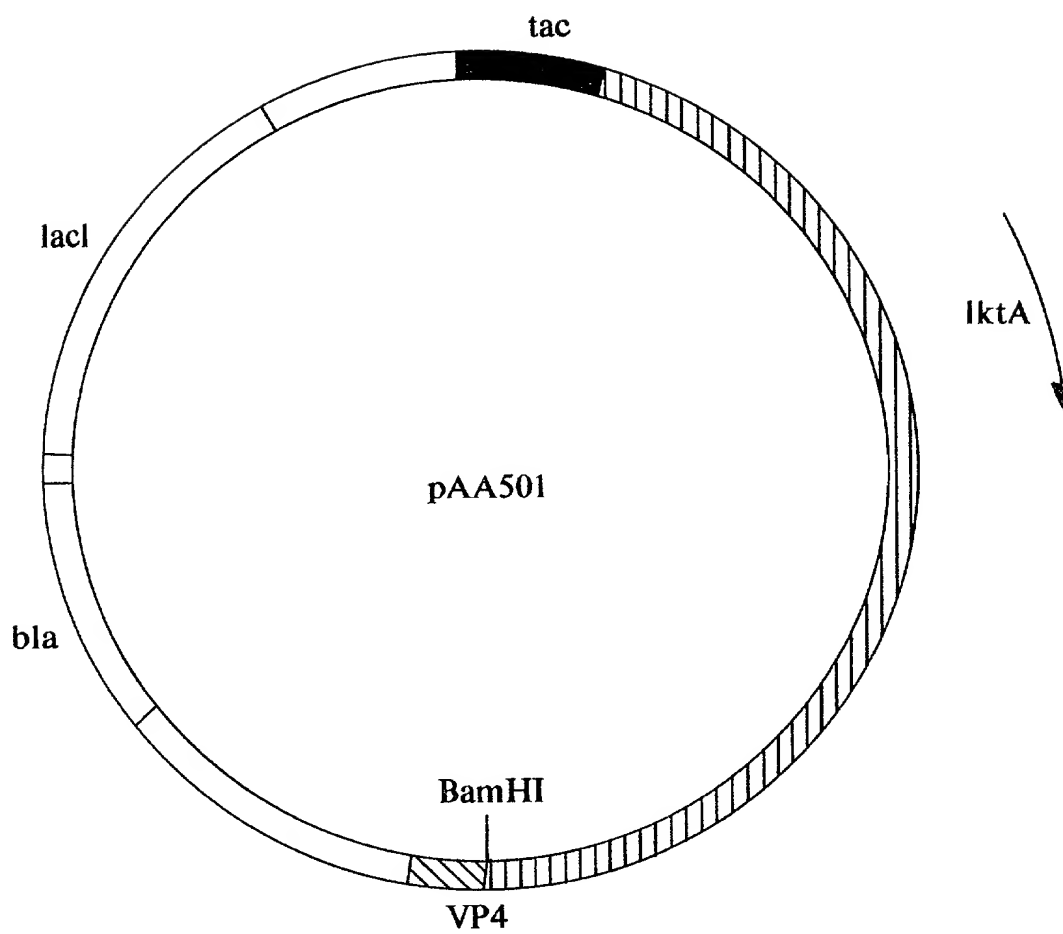


FIG. 9


```

      250      260      270      280
      *      *      *      *      *
TCC GCT CCA CAA ATT GAT AAA TTG CTA CAG AAA ACT AAA GCA GGC CAA
AGG CGA GGT GTT TAA CTA TTT AAC GAT GTC TTT TGA TTT CGT CCG GTT
Ser Ala Pro Gln Ile Asp Lys Leu Leu Gln Lys Thr Lys Ala Gly Gln>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

290      300      310      320      330
      *      *      *      *      *
GCA TTA GGT TCT GCC GAA AGC ATT GTA CAA AAT GCA AAT AAA GCC AAA
CGT AAT CCA AGA CCG CTT TCG TAA CAT GTT TTA CGT TTA TTT CCG TTT
Ala Leu Gly Ser Ala Glu Ser Ile Val Gln Asn Ala Asn Lys Ala Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      340      350      360      370      380
      *      *      *      *      *
ACT GTA TTA TCT GGC ATT CAA TCT ATT TTA GGC TCA GTA TTG GCT GGA
TGA CAT AAT AGA CCG TAA GTT AGA TAA AAT CCG AGT CAT AAC CGA CCT
Thr Val Leu Ser Gly Ile Gln Ser Ile Leu Gly Ser Val Leu Ala Gly>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      390      400      410      420      430
      *      *      *      *      *
ATG GAT TTA GAT GAG GCC TTA CAG AAT AAC AGC AAC CAA CAT GCT CTT
TAC CTA AAT CTA CTC CCG AAT GTC TTA TTG TCG TTG GTT GTA CGA GAA
Met Asp Leu Asp Glu Ala Leu Gln Asn Asn Ser Asn Gln His Ala Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      440      450      460      470      480
      *      *      *      *      *
GCT AAA GCT GGC TTG GAG CTA ACA AAT TCA TTA ATT GAA AAT ATT GCT
CGA TTT CGA CCG AAC CTC GAT TGT TTA AGT AAT TAA CTT TTA TAA CGA
Ala Lys Ala Gly Leu Glu Leu Thr Asn Ser Leu Ile Glu Asn Ile Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      490      500      510      520
      *      *      *      *      *
AAT TCA GTA AAA ACA CTT GAC GAA TTN -GT GAG CAA ATT AGT CAA TTT
TTA AGT CAT TTT TGT GAA CTG CTT AAN -CA CTC GTT TAA TCA GTT AAA
Asn Ser Val Lys Thr Leu Asp Glu Xxx Cys Glu Gln Ile Ser Gln Phe>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

530      540      550      560      570
      *      *      *      *      *
GGT TCA AAA CTA CAA AAT ATC AAA GGC TTA GGG ACT TTA GGA GAC AAA
CCA AGT TTT GAT GTT TTA TAG TTT CCG AAT CCC TGA AAT CCT CTG TTT
Gly Ser Lys Leu Gln Asn Ile Lys Gly Leu Gly Thr Leu Gly Asp Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

FIG. 10B

```

      580      590      600      610      620
      *      *      *      *      *
CTC AAA AAT ATC GGT GGA CTT GAT AAA GCT GGC CTT GGT TTA GAT GTT
GAG TTT TTA TAG CCA CCT GAA CTA TTT CGA CCG GAA CCA AAT CTA CAA
Leu Lys Asn Ile Gly Gly Leu Asp Lys Ala Gly Leu Gly Leu Asp Val>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      630      640      650      660      670
      *      *      *      *      *
ATC TCA GGG CTA TTA TCG GGC GCA ACA GCT GCA CTT GTA CTT GCA GAT
TAG AGT CCC GAT AAT AGC CCG CGT TGT CGA CGT GAA CAT GAA CGT CTA
Ile Ser Gly Leu Leu Ser Gly Ala Thr Ala Ala Leu Val Leu Ala Asp>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      680      690      700      710      720
      *      *      *      *      *
AAA AAT GCT TCA ACA GCT AAA AAA GTG GGT GCG GGT TTT GAA TTG GCA
TTT TTA CGA AGT TGT CGA TTT TTT CAC CCA CGC CCA AAA CTT AAC CGT
Lys Asn Ala Ser Thr Ala Lys Lys Val Gly Ala Gly Phe Glu Leu Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      730      740      750      760
      *      *      *      *
AAC CAA GTT GTT GGT AAT ATT ACC AAA GCC GTT TCT TCT TAC ATT TTA
TTG GTT CAA CAA CCA TTA TAA TGG TTT CGG CAA AGA AGA ATG TAA AAT
Asn Gln Val Val Gly Asn Ile Thr Lys Ala Val Ser Ser Tyr Ile Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

770      780      790      800      810
*      *      *      *      *
GCC CAA CGT GTT GCA GCA GGT TTA TCT TCA ACT GGG CCT GTG GCT GCT
CGG GTT GCA CAA CGT CGT CCA AAT AGA AGT TGA CCC GGA CAC CGA CGA
Ala Gln Arg Val Ala Ala Gly Leu Ser Ser Thr Gly Pro Val Ala Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      820      830      840      850      860
      *      *      *      *      *
TTA ATT GCT TCT ACT GTT TCT CTT GCG ATT AGC CCA TTA GCA TTT GCC
AAT TAA CGA AGA TGA CAA AGA GAA CGC TAA TCG GGT AAT CGT AAA CGG
Leu Ile Ala Ser Thr Val Ser Leu Ala Ile Ser Pro Leu Ala Phe Ala>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

FIG. 10C

870 880 890 900 910
 * * * * *
 GGT ATT GCC GAT AAA TTT AAT CAT GCA AAA AGT TTA GAG AGT TAT GCC
 CCA TAA CGG CTA TTT AAA TTA GTA CGT TTT TCA AAT CTC TCA ATA CGG
 Gly Ile Ala Asp Lys Phe Asn His Ala Lys Ser Leu Glu Ser Tyr Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

920 930 940 950 960
 * * * * *
 GAA CGC TTT AAA AAA TTA GGC TAT GAC GGA GAT AAT TTA TTA GCA GAA
 CTT GCG AAA TTT TTT AAT CCG ATA CTG CCT CTA TTA AAT AAT CGT CTT
 Glu Arg Phe Lys Lys Leu Gly Tyr Asp Gly Asp Asn Leu Leu Ala Glu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

970 980 990 1000
 * * * * *
 TAT CAG CGG GGA ACA GGG ACT ATT GAT GCA TCG GTT ACT GCA ATT AAT
 ATA GTC GCC CCT TGT CCC TGA TAA CTA CGT AGC CAA TGA CGT TAA TTA
 Tyr Gln Arg Gly Thr Gly Thr Ile Asp Ala Ser Val Thr Ala Ile Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1010 1020 1030 1040 1050
 * * * * *
 ACC GCA TTG GCC GCT ATT GCT GGT GGT GTG TCT GCT GCT GCA GCC GGC
 TGG CGT AAC CGG CGA TAA CGA CCA CCA CAC AGA CGA CGA CGT CGG CCG
 Thr Ala Leu Ala Ala Ile Ala Gly Gly Val Ser Ala Ala Ala Ala Gly>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1060 1070 1080 1090 1100
 * * * * *
 TCG GTT ATT GCT TCA CCG ATT GCC TTA TTA GTA TCT GGG ATT ACC GGT
 AGC CAA TAA CGA AGT GGC TAA CGG AAT AAT CAT AGA CCC TAA TGG CCA
 Ser Val Ile Ala Ser Pro Ile Ala Leu Leu Val Ser Gly Ile Thr Gly>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1110 1120 1130 1140 1150
 * * * * *
 GTA ATT TCT ACG ATT CTG CAA TAT TCT AAA CAA GCA ATG TTT GAG CAC
 CAT TAA AGA TGC TAA GAC GTT ATA AGA TTT GTT CGT TAC AAA CTC GTG
 Val Ile Ser Thr Ile Leu Gln Tyr Ser Lys Gln Ala Met Phe Glu His>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1160 1170 1180 1190 1200
 * * * * *
 GTT GCA AAT AAA ATT CAT AAC AAA ATT GTA GAA TGG GAA AAA AAT AAT
 CAA CGT TTA TTT TAA GTA TTG TTT TAA CAT CTT ACC CTT TTT TTA TTA
 Val Ala Asn Lys Ile His Asn Lys Ile Val Glu Trp Glu Lys Asn Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

FIG. 10D

08975566-113497

1210 1220 1230 1240
 * * * * *
 CAC GGT AAG AAC TAC TTT GAA AAT GGT TAC GAT GCC CGT TAT CTT GCG
 GTG CCA TTC TTG ATG AAA CTT TTA CCA ATG CTA CGG GCA ATA GAA CGC
 His Gly Lys Asn Tyr Phe Glu Asn Gly Tyr Asp Ala Arg Tyr Leu Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1250 1260 1270 1280 1290
 * * * * * *
 AAT TTA CAA GAT AAT ATG AAA TTC TTA CTG AAC TTA AAC AAA GAG TTA
 TTA AAT GTT CTA TTA TAC TTT AAG AAT GAC TTG AAT TTG TTT CTC AAT
 Asn Leu Gln Asp Asn Met Lys Phe Leu Leu Asn Leu Asn Lys Glu Leu>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1300 1310 1320 1330 1340
 * * * * * *
 CAG GCA GAA CGT GTC ATC GCT ATT ACT CAG CAG CAA TGG GAT AAC AAC
 GTC CGT CTT GCA CAG TAG CGA TAA TGA GTC GTC GTT ACC CTA TTG TTG
 Gln Ala Glu Arg Val Ile Ala Ile Thr Gln Gln Gln Trp Asp Asn Asn>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1350 1360 1370 1380 1390
 * * * * * *
 ATT GGT GAT TTA GCT GGT ATT AGC CGT TTA GGT GAA AAA GTC CTT AGT
 TAA CCA CTA AAT CGA CCA TAA TCG GCA AAT CCA CTT TTT CAG GAA TCA
 Ile Gly Asp Leu Ala Gly Ile Ser Arg Leu Gly Glu Lys Val Leu Ser>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1400 1410 1420 1430 1440
 * * * * * *
 GGT AAA GCC TAT GTG GAT GCG TTT GAA GAA GGC AAA CAC ATT AAA GCC
 CCA TTT CGG ATA CAC CTA CGC AAA CTT CTT CCG TTT GTG TAA TTT CGG
 Gly Lys Ala Tyr Val Asp Ala Phe Glu Glu Gly Lys His Ile Lys Ala>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

1450 1460 1470 1480
 * * * * * *
 GAT AAA TTA GTA CAG TTG GAT TCG GCA AAC GGT ATT ATT GAT GTG AGT
 CTA TTT AAT CAT GTC AAC CTA AGC CGT TTG CCA TAA TAA CTA CAC TCA
 Asp Lys Leu Val Gln Leu Asp Ser Ala Asn Gly Ile Ile Asp Val Ser>
 ___b___b___ RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>

FIG. 10E

08976566 "112497"

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1490      1500      1510      1520      1530
  *      *      *      *      *
AAT TCG GGT AAA GCG AAA ACT CAG CAT ATC TTA TTC AGA ACG CCA TTA
TTA AGC CCA TTT CGC TTT TGA GTC GTA TAG AAT AAG TCT TGC GGT AAT
Asn Ser Gly Lys Ala Lys Thr Gln His Ile Leu Phe Arg Thr Pro Leu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1540      1550      1560      1570      1580
  *      *      *      *      *
TTG ACG CCG GGA ACA GAG CAT CGT GAA CGC GTA CAA ACA GGT AAA TAT
AAC TGC GGC CCT TGT CTC GTA GCA CTT GCG CAT GTT TGT CCA TTT ATA
Leu Thr Pro Gly Thr Glu His Arg Glu Arg Val Gln Thr Gly Lys Tyr>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1590      1600      1610      1620      1630
  *      *      *      *      *
GAA TAT ATT ACC AAG CTC AAT ATT AAC CGT GTA GAT AGC TGG AAA ATT
CTT ATA TAA TGG TTC GAG TTA TAA TTG GCA CAT CTA TCG ACC TTT TAA
Glu Tyr Ile Thr Lys Leu Asn Ile Asn Arg Val Asp Ser Trp Lys Ile>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1640      1650      1660      1670      1680
  *      *      *      *      *
ACA GAT GGT GCA GCA AGT TCT ACC TTT GAT TTA ACT AAC GTT GTT CAG
TGT CTA CCA CGT CGT TCA AGA TGG AAA CTA AAT TGA TTG CAA CAA GTC
Thr Asp Gly Ala Ala Ser Ser Thr Phe Asp Leu Thr Asn Val Val Gln>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1690      1700      1710      1720
  *      *      *      *      *
CGT ATT GGT ATT GAA TTA GAC AAT GCT GGA AAT GTA ACT AAA ACC AAA
GCA TAA CCA TAA CTT AAT CTG TTA CGA CCT TTA CAT TGA TTT TGG TTT
Arg Ile Gly Ile Glu Leu Asp Asn Ala Gly Asn Val Thr Lys Thr Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1730      1740      1750      1760      1770
  *      *      *      *      *
GAA ACA AAA ATT ATT GCC AAA CTT GGT GAA GGT GAT GAC AAC GTA TTT
CTT TGT TTT TAA TAA CGG TTT GAA CCA CTT CCA CTA CTG TTG CAT AAA
Glu Thr Lys Ile Ile Ala Lys Leu Gly Glu Gly Asp Asp Asn Val Phe>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

1780      1790      1800      1810      1820
  *      *      *      *      *
GTT GGT TCT GGT ACG ACG GAA ATT GAT GGC GGT GAA GGT TAC GAC CGA
CAA CCA AGA CCA TGC TGC CTT TAA CTA CCG CCA CTT CCA ATG CTG GCT
Val Gly Ser Gly Thr Thr Glu Ile Asp Gly Gly Glu Gly Tyr Asp Arg>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

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FIG. 10F

1830	1840	1850	1860	1870
* * *	* * *	* * *	* * *	* * *
GTT CAC TAT AGC CGT GGA AAC TAT GGT GCT TTA ACT ATT GAT GCA ACC				
CAA GTG ATA TCG GCA CCT TTG ATA CCA CGA AAT TGA TAA CTA CGT TGG				
Val His Tyr Ser Arg Gly Asn Tyr Gly Ala Leu Thr Ile Asp Ala Thr>				
___b___b___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>				

1880	1890	1900	1910	1920
* * *	* * *	* * *	* * *	* * *
AAA GAG ACC GAG CAA GGT AGT TAT ACC GTA AAT CGT TTC GTA GAA ACC				
TTT CTC TGG CTC GTT CCA TCA ATA TGG CAT TTA GCA AAG CAT CTT TGG				
Lys Glu Thr Glu Gln Gly Ser Tyr Thr Val Asn Arg Phe Val Glu Thr>				
___b___b___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>				

1930	1940	1950	1960
* * *	* * *	* * *	* * *
GGT AAA GCA CTA CAC GAA GTG ACT TCA ACC CAT ACC GCA TTA GTG GGC			
CCA TTT CGT GAT GTG CTT CAC TGA AGT TGG GTA TGG CGT AAT CAC CCG			
Gly Lys Ala Leu His Glu Val Thr Ser Thr His Thr Ala Leu Val Gly>			
___b___b___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>			

1970	1980	1990	2000	2010
* * *	* * *	* * *	* * *	* * *
AAC CGT GAA GAA AAA ATA GAA TAT CGT CAT AGC AAT AAC CAG CAC CAT				
TTG GCA CTT CTT TTT TAT CTT ATA GCA GTA TCG TTA TTG GTC GTG GTA				
Asn Arg Glu Glu Lys Ile Glu Tyr Arg His Ser Asn Asn Gln His His>				
___b___b___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>				

2020	2030	2040	2050	2060
* * *	* * *	* * *	* * *	* * *
GCC GGT TAT TAC ACC AAA GAT ACC TTG AAA GCT GTT GAA GAA ATT ATC				
CGG CCA ATA ATG TGG TTT CTA TGG AAC TTT CGA CAA CTT CTT TAA TAG				
Ala Gly Tyr Tyr Thr Lys Asp Thr Leu Lys Ala Val Glu Glu Ile Ile>				
___b___b___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>				

2070	2080	2090	2100	2110
* * *	* * *	* * *	* * *	* * *
GGT ACA TCA CAT AAC GAT ATC TTT AAA GGT AGT AAG TTC AAT GAT GCC				
CCA TGT AGT GTA TTG CTA TAG AAA TTT CCA TCA TTC AAG TTA CTA CGG				
Gly Thr Ser His Asn Asp Ile Phe Lys Gly Ser Lys Phe Asn Asp Ala>				
___b___b___RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b___b___b___>				

FIG. 10G

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      2120      2130      2140      2150      2160
      *      *      *      *      *      *      *
TTT AAC GGT GGT GAT GGT GTC GAT ACT ATT GAC GGT AAC GAC GGC AAT
AAA TTG CCA CCA CTA CCA CAG CTA TGA TAA CTG CCA TTG CTG CCG TTA
Phe Asn Gly Gly Asp Gly Val Asp Thr Ile Asp Gly Asn Asp Gly Asn>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2170      2180      2190      2200
      *      *      *      *      *      *      *
GAC CGC TTA TTT GGT GGT AAA GGC GAT GAT ATT CTC GAT GGT GGA AAT
CTG GCG AAT AAA CCA CCA TTT CCG CTA CTA TAA GAG CTA CCA CCT TTA
Asp Arg Leu Phe Gly Gly Lys Gly Asp Asp Ile Leu Asp Gly Gly Asn>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2210      2220      2230      2240      2250
      *      *      *      *      *      *      *
GGT GAT GAT TTT ATC GAT GGC GGT AAA GGC AAC GAC CTA TTA CAC GGT
CCA CTA CTA AAA TAG CTA CCG CCA TTT CCG TTG CTG GAT AAT GTG CCA
Gly Asp Asp Phe Ile Asp Gly Gly Lys Gly Asn Asp Leu Leu His Gly>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2260      2270      2280      2290      2300
      *      *      *      *      *      *      *
GGC AAG GGC GAT GAT ATT TTC GTT CAC CGT AAA GGC GAT GGT AAT GAT
CCG TTC CCG CTA CTA TAA AAG CAA GTG GCA TTT CCG CTA CCA TTA CTA
Gly Lys Gly Asp Asp Ile Phe Val His Arg Lys Gly Asp Gly Asn Asp>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2310      2320      2330      2340      2350
      *      *      *      *      *      *      *
ATT ATT ACC GAT TCT GAC GGC AAT GAT AAA TTA TCA TTC TCT GAT TCG
TAA TAA TGG CTA AGA CTG CCG TTA CTA TTT AAT AGT AAG AGA CTA AGC
Ile Ile Thr Asp Ser Asp Gly Asn Asp Lys Leu Ser Phe Ser Asp Ser>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2360      2370      2380      2390      2400
      *      *      *      *      *      *      *
AAC TTA AAA GAT TTA ACA TTT GAA AAA GTT AAA CAT AAT CTT GTC ATC
TTG AAT TTT CTA AAT TGT AAA CTT TTT CAA TTT GTA TTA GAA CAG TAG
Asn Leu Lys Asp Leu Thr Phe Glu Lys Val Lys His Asn Leu Val Ile>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

      2410      2420      2430      2440
      *      *      *      *      *      *      *
ACG AAT AGC AAA AAA GAG AAA GTG ACC ATT CAA AAC TGG TTC CGA GAG
TGC TTA TCG TTT TTT CTC TTT CAC TGG TAA GTT TTG ACC AAG GCT CTC
Thr Asn Ser Lys Lys Glu Lys Val Thr Ile Gln Asn Trp Phe Arg Glu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

```

FIG. 10H

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2450      2460      2470      2480      2490
*          *          *          *          *
GCT GAT TTT GCT AAA GAA GTG CCT AAT TAT AAA GCA ACT AAA GAT GAG
CGA CTA AAA CGA TTT CTT CAC GGA TTA ATA TTT CGT TGA TTT CTA CTC
Ala Asp Phe Ala Lys Glu Val Pro Asn Tyr Lys Ala Thr Lys Asp Glu>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2500      2510      2520      2530      2540
*          *          *          *          *
AAA ATC GAA GAA ATC ATC GGT CAA AAT GGC GAG CGG ATC ACC TCA AAG
TTT TAG CTT CTT TAG TAG CCA GTT TTA CCG CTC GCC TAG TGG AGT TTC
Lys Ile Glu Glu Ile Ile Gly Gln Asn Gly Glu Arg Ile Thr Ser Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2550      2560      2570      2580      2590
*          *          *          *          *
CAA GTT GAT GAT CTT ATC GCA AAA GGT AAC GGC AAA ATT ACC CAA GAT
GTT CAA CTA CTA GAA TAG CGT TTT CCA TTG CCG TTT TAA TGG GTT CTA
Gln Val Asp Asp Leu Ile Ala Lys Gly Asn Gly Lys Ile Thr Gln Asp>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2600      2610      2620      2630      2640
*          *          *          *          *
GAG CTA TCA AAA GTT GTT GAT AAC TAT GAA TTG CTC AAA CAT AGC AAA
CTC GAT AGT TTT CAA CAA CTA TTG ATA CTT AAC GAG TTT GTA TCG TTT
Glu Leu Ser Lys Val Val Asp Asn Tyr Glu Leu Leu Lys His Ser Lys>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2650      2660      2670      2680
*          *          *          *          *
AAT GTG ACA AAC AGC TTA GAT AAG TTA ATC TCA TCT GTA AGT GCA TTT
TTA CAC TGT TTG TCG AAT CTA TTC AAT TAG AGT AGA CAT TCA CGT AAA
Asn Val Thr Asn Ser Leu Asp Lys Leu Ile Ser Ser Val Ser Ala Phe>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

2690      2700      2710      2720      2730
*          *          *          *          *
ACC TCG TCT AAT GAT TCG AGA AAT GTA TTA GTG GCT CCA ACT TCA ATG
TGG AGC AGA TTA CTA AGC TCT TTA CAT AAT CAC CGA GGT TGA AGT TAC
Thr Ser Ser Asn Asp Ser Arg Asn Val Leu Val Ala Pro Thr Ser Met>
__b__b__b__RECOMBINANT LEUKOTOXIN PEPTIDE [SPLIT]_b__b__b__>

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FIG. 10I

[illegible]

FIG. 10J